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(54) Hog cholera virus vaccine and diagnostic

Impfstoff und Diagnostikum für den Schweine-Cholera-Virus

Vaccin et test de diagnostic pour le virus du choléra porcin

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Description

[0001] The present invention is conc med with a nucleic acid sequence, a recombinant nucleic acid molecule comprising such a nucl ic acid sequence, a rec mbinant expression system comprising such a recombinant nucleic acid mol cule, a polypeptide characteristic of the hog cholera virus, a vaccine comprising such a polypeptide or recombinant expression system as well as a method for the preparation of such vaccines.

[0002] Classical swine fever or hog cholera (HC) represents an economically important disease of swine in many countries worldwide. Under natural conditions, the pig is the only animal known to be susceptible to HC. Hog cholera is a highly contagious disease which causes degeneration in the walls of capillaries, resulting in hemorrhages and necrosis of the internal organs. In the first instance hog cholera is characterized by fever, anorexia, vomiting and diarrhea which can be followed by a chronic course of the disease characterized by infertility, abortion and weak offsprings of sows. However, nearly all pigs die within 2 weeks after the first symptoms appear.

[0003] The causative agent, the hog cholera virus (HCV) has been shown to be structurally and serologically related to bovine viral diarrhea virus (BVDV) of cattle and to border disease virus (BDV) of sheep. These viruses are grouped together into the genus pestivirus within the family togaviridae. The nature of the genetic material of pestiviruses has long been known to be RNA. i.e. positive-strand RNA which lacks significant polyadenylation. The HCV probably comprises 3-5 structural proteins of which two are possibly glycosylated. The number of non-structural viral proteins is unknown.

[0004] Modified HCV vaccines (comprising attenuated or killed viruses) for combating HC infection have been developed and are presently used. However, infection of tissue culture cells to obtain HCV material to be used in said modified virus vaccines, leads to low virus yields and the virions are hard to purify. Modified live virus vaccines always involve the risk of inoculating animals with partially attenuated pathogenic HCV which is still pathogenic and can cause disease in the inoculated animal or offspring and of contamination by other viruses in the vaccine. In addition the attenuated virus may revert to a virulent state.

[0005] There are also several disadvantages using inactivated vaccines, e.g. the risk of only partial inactivation of viruses, the problem that only a low level of immunity is achieved requiring additional immunizations and the problem that antigenic determinants are altered by the inactivation treatment leaving the inactivated virus less immunogenic.

[0006] Furthermore, the usage of modified HCV vaccines is not suited for eradication programmes.

[0007] Until now, according to our knowledge diagnostic tests in swine which can distinguish between HCV or BVDV infection are not available. This is important as BVDV infection in pigs is of lower significance than HCV infection which means that BVDV infected pigs do not have to be eradicated.

[0008] Vaccines containing only the necessary and relevant HCV immunogenic material which is capable of eliciting an immune response against the pathogen do not display abovementioned disadvantages of modified vaccines.

[0009] According to the present invention a nucleic acid sequence which is a fragment of the hog cholera virus genome, and encoding a polypeptide characteristic of hog cholera virus which polypeptide comprises the amino acid sequence located within amino acid position 689-1067 has been found. Both the nucleic acid sequence and the polypeptide can be used for the preparation of a vaccine containing only the necessary and relevant immunogenic material for immunizing animals against HCV infection. "Nucleic acid sequence" refers both to a ribonucleic acid sequence and a deoxy-ribonucleic acid sequence.

[0010] A nucleic acid sequence according to the present invention is shown in figure 2. As is well known in the art, the degeneracy of the genetic code permits substitution of bases in a codon resulting in an other codon but still coding for the same amino acid, e.g. the codon for the amino acid glutamic acid is both GAT and GAA. Consequently, it is clear that for the expression of a polypeptide with the amino acid sequence shown in figure 2 encoded by a nucleic acid sequence according to the invention, use can be made of a nucleic acid sequence with such an alternative codon composition different from the nucleic acid sequence shown in figure 2.

[0011] Also included within the scope of the invention are nucleic acid sequences which hybridize under stringent conditions to the nucleic acid sequence shown in figure 2 encoding the polypeptide comprising the amino acid sequence located within amino acid position 689-1067. These nucleic acid sequences are related to the nucleic acid sequence shown in figure 2 but may comprise nucleotide substitutions, mutations, insertions, deletions etc. and encode polypeptides which are functionally equivalent to the polypeptide defined above shown in figure 2, i.e. the amino acid sequence of a related polypeptide is not identical with the amino acid sequence shown in figure 2 but features corresponding immunological properties characteristic for HCV.

[0012] The nucleic acid sequence shown in figure 2 is a cDNA sequence derived from the genomic RNA of HCV. This continuous sequence is 12284 nucleotides in length, and contains one long open reading frame (ORF), starting with the ATG codon at position 364 to 366 and ending with a TGA codon as a translational stop codon at position 12058 to 12060. This ORF consists of 3898 codons capable of encoding 435 kDa of protein.

[0013] In vivo, during HCV replication in an infected cell, this protein is synthesized as a polyprotein precursor molecule which is subsequently processed to fragment polypeptides by (enzymatic) cleavage of the precursor molecule.

These fragments form after possibl post-translational modifications the structural and non-structural proteins of the virus. The nucleic acid sequ nce according to the pres nt invention contains the genetic information for such a fragment with immunizing properties against HCV or immunological properties charact ristic for HCV or contains the gen tic information for a portion of such a fragment which still has the immunizing prop rties or the immunological properties charact ristic for HCV.

[0014] Fragment polypeptides of the polypeptide according to figure 2 and the portions thereof, which can be used for the immunisation of animals against HC or for diagnosis of HC are located within the amino acid position about 1-249, 263-487, 488-688 or 689-1067.

[0015] The 1-249 region essentially represents the core protein whereas the 263-487, 488-688 and 689-1067 regions essentially represent glycoproteins of 33 kD, 44/48 kD and 55 kD respectively. Within the scope of the invention is a nucleic acid sequences comprising the genetic information for at least the 55 kD coding region mentioned above or portions thereof.

[0016] A preferred region to be incorporated into a vaccine against HCV infection is the region corresponding to the 55 kD protein of HCV or a portion thereof still having immunizing activity.

[0017] Furthermore, a nucleic acid sequence at least comprising the coding sequences for said 55 kD protein or portion thereof can advantageously be applied according to the present invention.

[0018] In addition, a preferred portion of the HCV 55 kD protein, which can be used for immunization of pigs against HCV infection, is determined by analyses of HCV deletion mutants with anti-55 kD protein monoclonal antibodies having virus neutralizing activity. Such a portion comprising an epitope spans the amino acid sequence about 812-859 and is coded by the nucleotide sequence about 2799-2938. A nucleic acid sequence at least comprising said nucleotide sequence forms part of the present invention too.

[0019] A nucleic acid sequence according to the invention which can be used for the diagnosis of HCV infection in pigs and which can be applied to discriminate HCV from BVDV can be derived from the gene encoding the 55 kD protein.

[0020] Preferably, such a nucleic acid sequence is derived from the nucleotide sequences 2587-2619 or 2842-2880, both sequences being part of the gene encoding the 55 kD protein. A preferred oligonucleotide for diagnostic purposes is:

5' - CCT ACT AAC CAC GTT AAG TGC TGT GAC TTT AAA - 3'

or

5' - TTC TGT TCT CAA GGT TGT GGG GCT CAC TGC TGT GCA CTC - 3'

[0021] Moreover, a nucleic acid sequence comprising at least a sub-sequence of said oligonucleotides and which still can be used to differentiate between HCV and BVDV forms part of the invention.

[0022] The invention also relates to a test kit to be used in an assay, this test kit containing a nucleic acid sequence according to the invention.

[0023] Preferably the test kit comprises an oligonucleotide mentioned above or a nucleic acid sequence comprising at least a sub-sequence thereof.

[0024] Variations or modifications in the polypeptide shown in figure 2 or fragments thereof, such as natural variations between different strains or other derivatives, are possible while retaining the same immunologic properties. These variations may be demonstrated by (an) amino acid difference(s) in the overall sequence or by deletions, substitutions, insertions, inversions or additions of (an) amino acid(s) in said polypeptide.

[0025] Moreover, the potential exists, in the use of recombinant DNA technology, for the preparation of various derivatives of the polypeptide shown in figure 2 or fragments thereof, variously modified by resultant single or multiple amino acid substitutions, deletions, additions or replacements, for example by means of site directed mutagenesis of the underlying DNA. All nucleic acid sequences encoding such modifications resulting in derivatives of the polypeptide defined above shown in figure 2 or fragments thereof are included within the scope of the present invention so long as the essential characteristic activity of said polypeptide or fragment thereof, remains unaffected in essence.

[0026] RNA isolated from pelleted virions was isolated and used for the synthesis of cDNA. This cDNA was cloned in phage kgtII and the respective library was amplified and screened with goat anti-HCV antiserum. Two positive clones could be identified and shown to have inserts with sizes of 0,8 kb and 1,8 kb. The 0,8 kb kgtII insert was partially sequenced (see figure 3) and determined to be located between about 1,2 and 2,0 kb on the HCV genome (see figure 2).

[0027] The invention also relates to a test kit to be used in an assay, this test kit containing a nucleic acid sequence according to the invention.

[0028] A nucl ic acid sequence according to the present invention can be ligated to various vector nucleic acid

molecules such as plasmid DNA, bacteriophage DNA or viral DNA to form a recombinant nucleic acid molecule. The vector nucleic acid molecules preferably contain DNA sequ nces to initiate, control and terminate transcription and translation. A recombinant expression system comprising a host containing such a recombinant nucleic acid molecule can b used to allow for a nucleic acid sequence according to the present invention to express a polypeptid encoded by said nucleic acid sequence. The host of above-mentioned recombinant expression system can be of procaryotic origin, e.g. bacteria such as E.coli, B.subtilis and Pseudomonas, viruses such as vaccinia and fowl pox virus or eucaryotic origin such as yeasts or higher eucaryotic cells such as insect, plant or animal cells.

[0029] Immunization of animals against HC can, for example, be achieved by administering to the animal a polypeptide encoded by a nucleic acid sequence according to the invention as a so-called "sub-unit" vaccine. The subunit vaccine according to the invention comprises a polypeptide generally in a pure form, optionally in the presence of a pharmaceutically acceptable carrier.

[0030] Small fragments are preferably conjugated to carrier molecules in order to raise their immunogenicity. Suitable carriers for this purpose are macromolecules, such as natural polymers (proteins, like key hole limpet hemocyanin, albumin, toxins), synthetic polymers like polyamino acids (polylysine, polyalanine), or micelles of amphiphilic compounds like saponins. Alternatively these fragments may be provided as polymers thereof, preferably linear polymers. Polypeptides to be used in such subunit vaccines can be prepared by methods known in the art, e.g. by isolation said polypeptides from hog cholera virus, by recombinant DNA techniques or by chemical synthesis.

[0031] If required the polypeptides encoded by a nucleic acid sequence according to the invention to be used in a vaccine can be modified in vitro or in vivo, for example by glycosylation, amidation, carboxylation or phosphorylation.

[0032] An alternative to subunit vaccines are "vector" vaccines. A nucleic acid sequence according to the invention is integrated by recombinant techniques into the genetic material of another micro-organism (e.g. virus or bacterium) thereby enabling the micro-organism to express a polypeptide according to the invention. This recombinant expression system is administered to the animal to be immunized whereafter it replicates in the inoculated animal and expresses the polypeptide resulting in the stimulation of the immune system of the animal. Suitable examples of vaccine vectors are pox viruses (such as vaccinia, cow pox, rabbit pox), avian pox viruses (such as fowl pox virus) pseudorabies virus, adeno viruses, influenza viruses, bacteriophages or bacteria (such as Escherichia coli and Salmonella).

[0033] The recombinant expression system having a nucleic acid sequence according to the invention inserted in its nucleic acid sequence can for example be grown in a cell culture and can if desired be harvested from the infected cells and formed to a vaccine optionally in a lyophilized form. Said genetically manipulated micro-organism can also be harvested from live animals infected with said micro-organism. Abovementioned recombinant expression system can also be propagated in a cell culture expressing a polypeptide encoded by a nucleic acid sequence according to the invention, whereafter the polypeptide is isolated from the culture.

[0034] A vaccine comprising a polypeptide encoded by a nucleic acid sequence according to the invention or a recombinant expression system according to the present invention can be prepared by procedures well-known in the art for such vaccines. A vaccine according to the invention can consist inter alia of whole host, host extract, partially or completely purified polypeptide or a partially or completely purified recombinant expression system as above-mentioned.

[0035] The vaccine according to the invention can be administered in a conventional active immunization scheme: single or repeated administration in a manner compatible with the dosage formulation and in such amount as will be therapeutically effective and immunogenic. The administration of the vaccine can be done, e.g. intradermally, subcutaneously, intramusculary, intra-venously or intranasally. For parenteral administration the vaccines may additionally contain a suitable carrier, e.g. water, saline or buffer solution with or without adjuvants, stabilizers, solubilizers, emulsifiers etc.

[0036] The vaccine may additionally contain immunogens related to other diseases or nucleic acid sequences encoding these immunogens like antigens of parvovirus, pseudorabies virus, swine influenza virus, TGE virus, rotavirus, Escherichia coli, Bordetella, Pasteurella, Erysipelas etc. to produce a multivalent vaccine.

[0037] Polypeptides encoded by a nucleic acid sequence according to the present invention can also be used in diagnostic methods to detect the presence of HCV antigen or antibody in an animal. Moreover, nucleic acid sequences according to the invention can be used to produce polypeptides to be used in above-mentioned diagnostic methods or as a hybridisation probe for the detection of the presence of HCV nucleic acid in a sample.

Example 1

Immunological identification of cDNA clones

[0038] Infection of cells and harvesting of virus. PK15 and $38A_1D$ cells were grown in DMEM with 10% FCS and were infected in suspension by the virul nt HCV strain Alfort in a volume of 20-30 ml at a cell concentration of 5×10^7/ml at 37 °C for 90 min with an m.o.i. of 0.01 to 0.001 (as determined by immunofluorescence assay). Thereafter, the

PK15 cells were seeded in tissue culture plates (150 mm diameter), while the susp nsion cells $38A_1$ D wer incubated in bottles with gentle stirring (Tecnomara, Switzerland). For cDNA synthes , the tissu cultur supernatant was harv sted 48 hours aft r infection, clarified at 12,000 g, and afterwards the virus pellet d in a TFA 20 rotor (Contron, Italy) at 54,000 g for 12 hours.

Example 1

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[0039] Infection of cells and harvesting of virus. PK15 and $38A_1$ D cells were grown in DMEM with 10% FCS and were infected in suspension by the virulent HCV strain Alfort in a volume of 20-30 ml at a cell concentration of 5×10^7/ml at 37 °C for 90 min with an m.o.i. of 0.01 to 0.001 (as determined by immunofluorescence assay). Thereafter, the PK15 cells were seeded in tissue culture plates (150 mm diameter), while the suspension cells $38A_1$D were incubated in bottles with gentle stirring (Tecnomara, Switzerland). For cDNA synthese, the tissue culture supernatant was harvested 48 hours after infection, clarified at 12,000 g, and afterwards the virus pelleted in a TFA 20 rotor (Contron, Italy) at 54,000 g for 12 hours.

[0040] Preparation of goat anti-HCV serum. A fibroblastic cell strain was established from the skin biopsy of a young goat by standard cell culture techniques. The cells were initially grown in F-10 medium with 10% FCS and later in DMEM with 10% FCS. Goat fibroblasts were infected with HCV. Over the first 26 hours p.i., the cells were washed every 8 hours 3 times with PBS and afterwards incubated in DMEM with 10% preimmune goat serum (PGS). 48 hours p.i., the tissue culture supernatant was harvested and used as stock virus. Before immunization, goat cells for 30 tissue culture dishes (150 mm diameter) were kept for 3 passages in medium with 10% PGS and then infected with the stock virus. 48 hours p.i., the goat was immunized with X-ray-inactivated pelleted virus and infected cells. Both were emulsified in Freund's adjuvant (complete for basis immunization, incomplete for booster injections) and injected subcutaneously. To obtain antibodies recognizing denatured molecules, the antigen preparations were incubated in 0.2% SDS, 3 mM DTT at 95 °C for 5 min before injection.

[0041] RNA preparation, cDNA synthesis and cloning. RNA from virions was isolated by using the guanidine thiocyanate method described by Chirgwin et al. (1979). RNA from pelleted virions (5 µg total RNA, approximately 0.5 µg HCV RNA) and 0.1 µg of random hexanucleotide primer (Pharmacia, Sweden) in 20 µl of water were heated to 65 °C for 10 min, chilled on ice, and adjusted to first strand buffer (50 mM Tris-HCl pH 8.3; 30 mM KCl; 8 mM $MgCl_2$; 1 mM DTT, dATP, dCTP, dGTP, dTTP 1 mM each and 500 units RNAguard [Pharmacia, Sweden] per ml) in a final volume of 32 µl. 35 units of AMV reverse transcriptase (Life Sciences Inc., USA) were added. After 1 hour at 43 °C the reaction mixture was added to one vial of second strand synthesis mixture (cDNA synthesis kit, Pharmacia, Sweden). Second strand synthesis, preparation of blunt ends, and Eco RI adaptor ligation and phosphorylation were done as recommended by the supplier.

[0042] The cDNA was size-fractionated by preparative agarose gel electrophoresis. The part of the gel containing DNA molecules smaller than 0.5 kb was discarded. The remaining DNA was concentrated by running the gel reversely for 15 min and extracted from the agarose after 3 cycles of freezing and thawing with phenol.

[0043] Ethanol co-precipitated cDNA and λgt11 DNA (1 µg EcoRI digested dephosphorylated arms, Promega, USA) was ligated by 3 units of T4 DNA ligase (Pharmacia, Sweden) in a total volume of 10 ul ligase buffer (30 mM Tris-HCl pH 7.4; 10 mM $MgCl_2$; 10 mM DTT; 1 mM ATP). In vitro packaging with a commercially available extract (Packagene, Promega, USA) and infection of E.coli K12 cells, strain Y 1090, with resulting phages was performed as recommended by the supplier. The library was amplified once as described (Davis et al., 1986).

[0044] Screening of λgt11 library. Screening was basically performed as described (Young and Davis, 1983) using the Protoblot system purchased from Promega, USA (Huynh et al., 1985) and a serum dilution of 10^{-3}. For background reduction the goat anti HCV serum was treated with E.coli lysate (strain Y1090) at 0.8 mg/ml (Huynh et al., 1985). Two positive clones having inserts of 0.8 kb and 1.8 kb, respectively could be identified.

[0045] Nick translation and Northern hybridization. 50 ng of the 0.8 kb HCV nucleic acid sequence labeled with [α^{32}P] dCTP (3000 Ci per mMole, Amersham Buchler, FRG) by nick translation (nick translation kit, Amersham Buchler, FRG) was hybridized to Northern filters at a concentration of 5 ng per ml of hybridization mixture (5 x SSC; 1 x Denhardt's; 20 mM sodium phosphate pH 6.8; 0.1% SDS and 100 µg yeast tRNA [Boehringer-Mannheim, FRG] per ml) at 68 C for 12 to 14 hours. Membranes were then washed as described (Keil et al., 1984) and exposed at -70 °C to Kodak X-Omat AR films for varying times using Agfa Curix MR 800 intensifying screens.

[0046] The 0.8 kb nucleic acid sequence hybridized not only to intact HCV RNA but also to degradation products thereof. The 0.8 kb nucleic acid s quence did not hybridize to the 1.8 kb nucleic acid sequence, indicating that these two nucleic acid sequences correspond with fragments of th HCV genome which are not located in th same region of the genomic RNA.

[0047] Nucleotide sequencing. Subcloning of HCV specific phage DNA inserts into plasmid pEMBL 18 plus was done

according to standard procedures (Maniatis t al., 1982). Single-stranded DNA of recombinant pEMBL plasmids was prepared as described (Dent et al., 1985). Dideoxy sequ ncing reactions (Sanger et al., 1977) were carried out as recomm nded by the suppli r (Pharmacia, Sweden).

Example 2

Molecular cloning and nucleotide sequence of the genome of HCV

[0048] RNA preparation, cDNA synthesis and cloning. RNA preparation, cDNA synthesis, size selection and ligation of co-precipitated cDNA and λgt10 DNA (1 µg EcoRI digested dephosphorylated arms, Promega, USA) were done as described above. In vitro packaging of phage DNA using Packagene (Promega, USA) and titration of phages on E.coli strain C 600 HFL were performed as suggested by the supplier. The library was amplified once (Davis et al., 1986), and replicas transferred to nictrocellulose membranes (Amersham Buchler, FRG) (Benton and Davis, 1977) were hybridized with oligonucleotides as described above for Northern hybridization. Screening with cDNA fragments labeled with [α^{32}P] dCTP by nick translation (nick translation kit, Amersham Buchler, FRG) was done as described by Benton and Davis (1977). Positive clones were plaque purified and inserts subcloned into pEMBL plasmids (Maniatis et al., 1982; Dente et al., 1985; Davis et al., 1986).

[0049] A ^{32}P 5'-end labeled oligonucleotide of 17 bases complementary to the RNA sequence encoding the amino acid sequence Cys Gly Asp Asp Gly Phe was used for screening a λgt10 cDNA library. This oligonucleotide which hybridized to the about 12 kb genomic RNA of HCV, identified inter alia a clone with an insert of 0.75 kb, which hybridized also to HCV RNA. This 0.75 kb nucleic acid sequence which represents a fragment of the HCV genome together with the 0.8 kb λgt11 nucleic acid sequence insert were used for further library screening resulting in a set of overlapping HCV nucleic acid sequences of which the relative positions and restriction site maps are shown in figure 1. These nucleic acid sequence fragments of the HCV genome are located between the following nucleic acid positions

4.0 kb fragment	27-4027
4.5 kb fragment	54-4494
0.8 kb fragment	1140-2002
4.2 kb fragment	3246-7252
5.5 kb fragment	6656-11819

and within about the following nucleic acid positions

3.0 kb fragment	8920-11920
1.9 kb fragment	10384-12284
0.75 kb fragment	10913-11663

[0050] Nucleotide sequencing. For complete nucleotide sequence determination exonuclease III and nuclease S1 (enzymes from Boehringer Mannheim, FRG) were used to establish deletion libraries of HCV derived cDNA inserts subcloned into pEMBL 18+ or 19+ plasmids (Hennikoff, 1987). Dideoxy sequencing (Sanger et al. 1977) of single stranded (Dente et al., 1985) or double stranded DNA templates was carried out using the T7 polymerase sequencing kit (Pharmacia, Sweden).

[0051] From the cDNA fragments a continuous sequence of 12284 nucleotides in length could be determined as shown in figure 2. This sequence contains one long open reading frame (ORF), starting with the ATG codon at position 364 to 366 and ending with TGA as a translational stop codon at 12058 to 12060. This ORF consists of 3898 codons capable of encoding a 435 kDa protein with an amino acid sequence shown in figure 2. Three nucleotide exchanges were detected as a result of differences in nucleotide sequence caused by possible heterogenicity of the virus population, two of which resulted in changes in the deduced amino acid sequence (figure 2).

[0052] It is concluded that almost the complete HCV genome has been cloned and sequenced by the procedures described above.

[0053] The 0.8 kb λgt11 nucleic acid sequence encoding an immunogenic HCV polypeptide identified with anti HCV serum was partially sequenced (see figure 3) which revealed that this sequence is located within 1.2 and 2.0 kb on the HCV RNA.

Example 3

Molecular cloning and xpr ssion of fusion prot ins of HCV

[0054] cDNA fragments derived from two regions of the HCV genome, i.e. the 0,8 kb λgt11 insert of exampl 1 encoding amino acids 262-546 (see figure 2) and the nucleic acid sequence encoding amino acids 747-1071 (figure 2), are expressed as fusion proteins in the pEx system (Strebel, K. et al., 1986).

[0055] Bacterial extracts were separated by SDS-PAGE and stained according to standard procedures, and then tested for reactivity with the goat anti-HCV serum of example 1 in a Western blot.

[0056] The HCV specific fusion proteins were partially purified by SDS-PAGE and transfered to nitrocellulose and incubated with the goat anti-HCV serum. Specific antibodies against said fusion proteins were obtained after elution.

[0057] Antibodies specific for the above-mentioned fusion proteins were employed in a radio-immuno precipitation assay.

Results

[0058] Both fusion proteins expressed in the pEx system were clearly identified as HCV specific after reaction with the goat anti-HCV serum.

[0059] Monospecific antiserum prepared against both fusions proteins precipitated HCV glycoproteins.

[0060] Antibodies specific for the 262-546-fusion protein precipitated the 44/48 kD and 33 kD protein, antibodies specific for the 747-1071-fusion protein precipitated the 55 kD protein from virus infected cells.

Example 4

Molecular cloning and expression of structural proteins via vaccinia virus

[0061] A fragment of the 4,0 kb clone shown in figure 1 (pHCK11) is prepared starting at the Hinfl restriction site (nucleotide 372) and ending at an artificial EcoRI site (nucleotide 4000) (Maniatis et al. 1982). For the 5' end an oligo-nucleotide adaptor was synthesized which contained an overhang compatible to BamHI, the original ATG(364-366) as translational start codon and a protruding end compatible to Hinfl at the 3' end.

```
5' GATCCACCATGGAGTT          HinfI
BamHI      GTGGTACCTCAACTTA  5'
```

At the 3' end of the construct a translational stop codon was introduced by deletion of the EcoRI protruding end with Mung bean nuclease and ligation into a blunt-end StuI/EcoRI adaptor residue:

```
5' GCCTGAATTC      3'EcoRI
   CGGACTTAAG
```

(Maniatis et al. 1982).

[0062] Prior to inserting above-mentioned HCV sequences into vaccinia virus the heterologous gene is cloned into a recombination vector. For this purpose a pGS62 plasmid (Cranage, M.P. et al. 1986) was used which contains a cloning site downstream the P7.5K promotor within the 4.9kb thymidine kinase sequence. The cloning site comprises three unique restriction sites, BamHI, SmaI and EcoRI. The recombination vector pGS62-3.8 was established by ligation of the described HCV sequence (372-4000) together with the adaptors into the BamHI/EcoRI digested pGS62.

[0063] Based on the plasmid a set of 15 deletion mutants was established. By treatment with ExonucleaseIII (Hennikof et al., 1987) subsequent shortening of the HCV cDNA from the 3' end was performed. All deletions are located within the region coding for the HCV 55 kD protein by removal of about 100bp; most of the 55 kD protein is lost in mutant 15 ending at nucleotide 2589. ExoIII shortened cDNA clones were ligated into the pGS62 giving rise to pGS62-3.8Exo 1-15 (figure 4).

[0064] CVI cells were infected with vaccinia (strain Copenhagen, mutant TS7) at a MOI of 0.1. Three hours after infection pGS62-3.8 DNA as well as vaccinia WR DNA were transfected by the Ca3(PO<)2 precipitation method and incubated for two days. Virus progeny was harvested and selected for tk-phenotyp on 143 tk-cells in th presence of

brom-deoxy-Uridine (100 µg/ml). This selection was performed at least twice followed by two further cycles of plaque purification.

Characterization of vaccinia-HCV recombinants

[0065] CVI cells were infected at an MOI between 2 and 10 with vaccinia-HCV recombinants and incubated for 8-16 hours. After fixation of the cells indirect immunofluorescence was performed using either monoclonal antibodies specific for HCV 55 kD protein or polyvalent anti-HCV sera. In all cases a cytoplasmatic fluorescence could be demonstrated.

[0066] After radioimmunoprecipitation and western blot analysis of cells infected with vaccinia recombinants four HCV-specific proteins were detected. By labeling with [^{3}H] glucosamine it was shown that three of these proteins are glycosylated. The apparent molecular weights of these proteins were identical to those found in HCV infected cells with HCV specific sera, namely 20 kD(core), 44/48 kD, 33 kD and 55 kD.

[0067] Proteolytic processing and modifications appear to be authentic since HCV proteins produced by expression via vaccinia virus have the same apparent molecular weights as in HCV infected cells.

Induction of neutralizing antibodies against HCV in mice.

[0068] Four groups of mice (3 mice/group) were infected once with

a.	Vaccinia WR wildtype	(5x10^6 pfu/individual)	**WR**
b.	Vaccinia 3.8 recombinant	(5x10^7 pfu/individual)	**VAC3.8**
c.	Vaccinia 3.8Exo 4 (55 kD deleted)	(5x10^7 pfu/individual)	**VAC3.8Exo 4**
d.	Vaccinia 3.8Exo 5	(5x10^7 pfu/individual)	**VAC3.8Exo 5**
e.	Vaccinia 3.8Exo 15 (55 kD deleted)	(5x10^7 pfu/individual)	**VAC3.8Exo 15**

by injection of purified virus intraperitoneally.
Mice were bled three weeks later. The reactivity of the sera was checked in a virus neutralization assay with HCV (Alfort) on PK[15] cells after serial dilution. (Rümenapf, T. et al. 1989).

Neutralization titers		
a.	WR	<1:2
b.	VAC3.8	1:96
	c. VAC3.8Exo 4	1:96
d.	VAC3.8Exo 5	<1:2
e.	VAC3.8Exo 15	<1.2

[0069] From the above it can be concluded that vaccinia virus containing a nucleic acid sequence comprising the genetic information for all structural proteins (VAC3.8) is able to induce virus neutralizing antibodies in mice, while incomplete constructs VAC3.8Exo 5-15 and WR are not.

[0070] As all deletions are located within the region coding for HCV 55 kD protein (most of the 55 kD protein is lost in mutant 15 ending at nucleotide 2589) and the other structural proteins are still being expressed by the recombinant vaccinia virus, it is clear that the 55 kD protein is responsible for the induction of HCV neutralizing antibodies.

Example 5

Immunization of pigs with VAC3.8

[0071] Out of three piglets (about 20 kg in weight) one animal (no. 28) was infected with wild type vaccinia virus (WR strain) and the other two (no. 26, 27) with recombinant VAC3.8 (i.p., i.v. and i.d., respectively). For infection 1x10^8 pfu of vaccinia virus is applied to each animal.

[0072] Clinical signs in the course of vaccinia infection were apparent as erythema at the side of scarification and fever (41 °C) at day six after infection.

Titers against vaccinia and hog cholera virus:

[0073] Thre weeks after infection the r activity of the respective sera against vaccinia (WR on CVI cells) and HCV (Alfort on PK15 cells) was checked.

[0074] Neutralization was assayed after s rial dilution of the sera by checking for complete absence of cpe (vaccinia)

or specific signals in immunofluoresc nce (HCV). (Rümenapf, T. et al. 1989).

Neutralization titers against vaccinia:	
pig 28 (WR)	1:8
pig 26 (VAC3.8)	1:16
pig 27 (VAC3.8)	1:16

Neutralization titers against HCV:	
pig 28 (WR)	<1:2
pig 26 (VAC3.8)	1:32
pig 27 (VAC3.8)	1:16

Challenge with HCV:

[0075] Four weeks after immunization with vaccinia each of the pigs was challenged by infection with $5x10^7$ $TCID_{50}$ HCV Alfort. Virus was applicated oronasal according to the natural route of infection. This amount of virus has been experimentally determined to be compulsory lethal for pigs.

[0076] On day five after the challenge infection pig 28 revealed fever of 41.5 °C and kept this temperature until day 12. The moribund animal was killed that day expressing typical clinical signs of acute hog cholera.

[0077] Both pigs (26, 27) immunized with VAC3.8 did not show any sign of illness after the challenge with HCV for more than 14 days.

Example 6

Construction of a 55 kD protein expression vector

[0078] Clone pHCK11 is digested with restriction enzymes SacI and HpaI according to standard techniques. The resulting 1.3 kb fragment, located between nucleotides 2672 (AGCTC) and 3971 (GTT) comprising most of HCV 55 kD protein, is isolated and cloned into the pseudorabies virus (PRV) gX gene (Maniatis et al. 1982).

[0079] Briefly, the cloned gX sequence was digested with SacI and ApaI. The ApaI 5' protruding ends were made blunt by filling up with Klenow fragment. After ligation the putative gX leader peptide coding sequence was located just upstream of the inserted HCV 55 kD sequence.

[0080] A translational stop codon downstream the HCV sequence was introduced by digestion with Bgl II (Bgl II site: 3936-3941) and religation after filling up the overhangs with Klenow fragment. This construct was placed downstream of the PRV gX promotor (clone 16/4-1.3). Clone 16/4-1.3 was transfected into MDBK cells by the DEAE dextran method (Maniatis et al. 1989). 16 h. later cells were infected with PRV (m.o.i. = 1). 4 h. post infection cells were fixed with a mixture of cold (-20 C) methanol/acetone. Indirect immunofluorescence with monoclonal antibodies (MABs) anti-HCV 55 kD protein revealed a specific signal in 5-10% of the cells. PRV infected cells without transfection and cells only transfected with clone 16/4-1.3 did not show any signal in this assay.

Brief description of the drawings

[0081] Fig. 1 displays physical maps of different HCV derived cDNA clones and their position relative to the RNA genome (upper line). Two HCV derived cDNA clones isolated after screening with either the antibody probe (0.8 kb clone) or the degenerated oligonucleotide probe (0.75 kb clone) are shown in the second line. The cDNA fragments chosen for nucleotide sequencing are indicated below. All numbers represent sizes of DNA fragments in kb. Restriction sites: B = Bgl II; E = EcoRI; H = Hind III; K = Kpn I; S = Sal I; Sm = Sma I.

[0082] Fig. 2 depicts a nucleic acid sequence of HCV and deduced amino acid sequence of the long open reading frame. Nucleotide exchanges between different cDNA clones and resulting changes in amino acid sequence are indicated. The part of the sequence corresponding to the oligonucleotide used for screening is underlined.

[0083] Fig. 3 shows the cDNA sequence from part of the 0.8 kb HCV insert of a λgt11 clone and the deduced amino acid sequence in one-letter code.

[0084] Fig. 4 shows the length of th HCV DNA cloned in the pGS62 vector. A set of 15 deletion mutants derived from cDNA clone pHCK11 was established by treatment with Exonuclease III and cloned in the pGS62 vector giving rise to pGS62-3.8Exo 1-15. 3' end nucleotides are indicated.

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Claims

Claims for the following Contracting States : BE, CH, DK, FR, GB, IT, LI, NL, SE

1. A nucleic acid sequence corresponding to a fragment of the hog cholera virus genome, and encoding a hog cholera virus polypeptide or an antigenic fragment thereof, capable of inducing virus neutralising antibodies, which polypeptide comprises the amino acid sequence located within the amino acid position 689-1067 shown in Fig 2 or is a functional equivalent thereof.

2. A nucleic acid sequence according to claim 1 characterised in that it comprises either the nucleotide sequence 2428-3564 shown in Fig 2 or a fragment thereof, or a nucleotide sequence which hybridises under stringent conditions to said nucleotide sequence shown in Fig 2.

3. A recombinant nucleic acid molecule comprising a vector nucleic acid molecule and a nucleic acid sequence according to claim 1 or claim 2.

4. A host cell capable of producing a polypeptide of hog cholera virus comprising a recombinant nucleic acid molecule according to claim 3.

5. A host cell according to claim 4 characterised in that the host cell is a bacterium.

6. A recombinant virus containing a recombinant nucleic acid molecule according to claim 3.

7. A method for producing a hog cholera virus polypeptide which polypeptide corresponds to a portion of the hog cholera virus precursor polyprotein, capable of inducing virus neutralising antibodies, wherein a host cell according to claim 4 or 5, or a recombinant virus according to claim 6 is propagated in a culture under conditions whereby the polypeptide is expressed, wh reaft r the polypeptide is isolated from the culture.

8. A vaccine for the protection of animals against hog cholera virus infection, characterised in that it comprises a hog cholera virus polypeptide which polyp ptide corresponds to a portion of the hog cholera virus precursor polyprotein, capable of inducing virus neutralising antibodies, which polypeptide comprises the amino acid sequ nce located within th amino acid position 689-1067 shown in Fig 2 *in non-denatured and non-reduced form,* or is an antigenic fragment or functional equivalent thereof.

9. A vaccine for the protection of animals against hog cholera virus infection, characterised in that it comprises a hog cholera virus polypeptide *as obtainable by expression in a recombinant host cell of* a nucleic acid sequence corresponding to a fragment of the hog cholera virus genome, and encoding a hog cholera virus polypeptide or an antigenic fragment thereof, capable of inducing virus neutralising antibodies, which polypeptide comprises the amino acid sequence located within the amino acid position 689-1067 shown in Fig 2 or is a functional equivalent thereof.

10. A vaccine according to claim 9, wherein said nucleic acid sequence comprises either the nucleotide sequence 2428-3564 shown in Fig 2 or a fragment thereof, or a nucleotide sequence which hybridises under stringent conditions to said nucleotide sequence shown in Fig 2.

11. A vaccine for the protection of animals against hog cholera virus infection, characterised in that it comprises a host cell according to claims 4-5 or a recombinant virus according to claim 6.

12. A method for the preparation of a hog cholera virus vaccine, characterised in that a polypeptide as defined in claims 8-10 is formed to a pharmaceutical preparation with immunising activity.

13. A method for the preparation of a hog cholera vaccine, characterised in that a host cell according to claims 4-5 or a recombinant virus according to claim 6 is propagated in a culture, whereafter the host cell or the recombinant virus is harvested and is formed to a pharmaceutical preparation with immunising activity.

Claims for the following Contracting States : ES, GR

1. A process for the preparation of a recombinant nucleic acid molecule which comprises incorporating a nucleic acid sequence corresponding to a fragment of the hog cholera virus genome, and encoding a hog cholera virus polypeptide or an antigenic fragment thereof, capable of inducing virus neutralizing antibodies, which polypeptide comprises the amino acid sequence located within the amino acid position 689-1067 shown in figure 2 or is a functional equivalent thereof, in a vector nucleic acid molecule.

2. A process according to claim 1, characterized in that said nucleic acid sequence comprises, either the nucleotide sequence 2428-3564 shown in figure 2 or a fragment thereof, or a nucleotide sequence which hybridizes under stringent conditions to said nucleotide sequence shown in figure 2.

3. A process which comprises expressing in a host cell the nucleic acid sequence defined in claim 1 or 2.

4. A process according to claims 3, characterized in that the host cell is a bacterium.

5. A process for the preparation of a recombinant virus, characterized in that the nucleic acid sequence defined in claim 1 or 2 is incorporated into a virus.

6. A process according to claims 3 or 4 which comprises allowing expression of the nucleic acid sequence in a culture, and recovering the polypeptide from the culture.

7. A process for the preparation of a vaccine for the protection of animals against hog cholera virus infection comprising, following the process of claim 6, the forming of the polypeptide expression product to a pharmaceutical preparation with immunizing activity.

8. A process for the preparation of a vaccine for the protection of animals against hog cholera virus infection comprising forming a host cell capabl of expressing the nucleic acid sequence defined in claims 1 or 2, or a recombinant virus comprising th nucleic acid sequence defin d in claims 1 or 2, to a pharmaceutical preparation with immunizing activity.

Patentansprüche

Patentansprüche für folgende Vertragstaaten: BE, CH, DE, DK, FR, GB, IT, LI, NL, SE

1. Nukleinsäuresequenz, die einem Fragment des Schweinepestvirusgenoms entspricht und ein Schweinepestvirus-Polypeptid oder antigenisches Fragment desselben codiert, das dazu fähig ist, virusneutralisierende Antikörper zu induzieren, wobei das Polypeptid die in der in Figur 2 gezeigten Aminosäureposition 689-1067 vorliegende Aminosäuresequenz umfaßt oder ein funktionelles Äquivalent davon ist.

2. Nukleinsäuresequenz gemäß Anspruch 1, dadurch gekennzeichnet, daß sie entweder die in Figur 2 dargestellte Nukleotidsequenz 2428-3564 oder ein Fragment derselben oder eine Nukleotidsequenz, die unter strengen Bedingungen mit der in Figur 2 dargestellten Nukleotid-sequenz hybridisiert, enthält.

3. Rekombinantes Nukleinsäuremolekül mit einem Vektomukleinsäuremolekül und einer Nukleinsäuresequenz gemäß Anspruch 1 oder 2.

4. Wirtszelle, die dazu fähig ist, ein Schweinepestvirus-Polypeptid mit einem rekombinanten Nukleinsäuremolekül gemäß Anspruch 3 zu produzieren.

5. Wirtszelle gemäß Anspruch 4, dadurch gekennzeichnet, daß es sich dabei um ein Bakterium handelt.

6. Rekombinantes Virus mit einem rekombinanten Nukleinsäuremolekül gemäß Anspruch 3.

7. Verfahren zur Herstellung eines Schweinepestvirus-Polypeptids, das einem Teil des Schweinepestvirus-Vorläuferpolyproteins entspricht und dazu fähig ist, virusneutralisierende Antikörper zu induzieren, bei dem eine Wirtszelle gemäß Anspruch 4 oder 5 oder ein rekombinantes Virus gemäß Anspruch 6 in einer Kultur unter Bedingungen, unter denen das Polypeptid expremiert wird, gezüchtet wird, wonach man das Polypeptid aus der Kultur isoliert.

8. Impfstoff zum Schutz von Tieren gegen Infektion mit Schweinepestvirus, dadurch gekennzeichnet, daß er ein Schweinepestvirus-Polypeptid enthält, das einem Teil des Schweinepestvirus-Vorläuferpolyproteins entspricht und dazu fähig ist, virusneutralisierende Antikörper zu induzieren, wobei das Polypeptid die innerhalb der in Figur 2 dargestellten Aminosäureposition 689-1067 befindliche Aminosäuresequenz *in nichtdenaturierter und nichtreduzierter Form* enthält oder ein antigenisches Fragment oder funktionelles Äquivalent davon ist.

9. Impfstoff zum Schutz von Tieren gegen Infektion mit Schweinepestvirus, dadurch gekennzeichnet, daß er ein Schweinepestvirus-Polypeptid in der Form enthält, *wie es durch Expression in einer rekombinanten Wirtszelle einer* Nukleinsäuresequenz *erhalten werden kann,* die einem Fragment des Schweinepestvirus-Genoms entspricht und ein Schweinepestvirus-Polypeptid oder antigenes Fragment davon codiert, das fähig ist, virusneutralisierende Antikörper zu induzieren, wobei das Polypeptid die innerhalb der in Figur 2 dargestellten Aminosäureposition 689-1067 befindliche Aminosäuresequenz umfaßt oder ein funktionelles Äquivalent davon ist.

10. Impfstoff gemäß Anspruch 9, wobei die Nukleinsäuresequenz entweder die in Figur 2 dargestellte Nukleotidsequenz 2428-3564 bzw. ein Fragment davon oder eine Nukleotidsequenz, die unter strengen Bedingungen mit der in Figur 2 dargestellten Nukleotidsequenz hybridisiert, enthält.

11. Impfstoff zum Schutz von Tieren gegen Infektion mit Schweinepestvirus, dadurch gekennzeichnet, daß er eine Wirtszelle gemäß Ansprüchen 4-5 oder ein rekombinantes Virus gemäß Anspruch 6 enthält.

12. Verfahren zur Herstellung eines Schweinepestvirus-Impfstoffs, dadurch gekennzeichnet, daß ein wie in den Ansprüchen 8-10 definiertes Polypeptid als pharmazeutische Zusammensetzung mit immunisierender Wirksamkeit formuliert wird.

13. Verfahren zur Herstellung eines Schweinepest-Impfstoffs, dadurch gekennzeichnet, daß eine Wirtszelle gemäß den Ansprüchen 4-5 oder ein rekombinantes Virus gemäß Anspruch 6 in einer Kultur gezüchtet wird, wonach die Wirtszelle bzw. das rekombinante Virus geemtet wird und als pharmazeutische Zusammensetzung mit immunisierender Wirksamkeit formuliert wird.

Patentansprüche für folgende Vertragsstaaten : ES, GR

1. Ein Verfahren zur Herstellung eines rekombinanten Nukleinsäuremoleküls, welches die Einverleibung einer Nukleinsäuresequenz entsprechend einem Fragment des Schweinepestvirusgenoms und das Codieren eines Schweinepestviruspolypeptids oder eines antigenen Fragmentes davon, das fähig ist, virusneutralisierende Antikörper zu induzieren, wobei das Polypeptid die innerhalb der in Figur 2 dargestellten Aminosäurestellung 689-1067 liegende Aminosäuresequenz umfasst oder ein funktionelles Aequivalent davon ist, in ein Vektornukleinsäuremolekül umfasst.

2. Ein Verfahren nach Anspruch 1, dadurch gekennzeichnet, dass die genannte Nukleinsäuresequenz entweder die in Figur 2 dargestellte Nukleotid-Sequenz 2428-3564 oder ein Fragment davon oder eine Nukleotid-Sequenz, welche unter stiringenten Bedingungen zu der genannten, in Figur 2 dargestellten Nukleotidsequenz hybridisiert, umfasst.

3. Ein Verfahren, welches die Expression der in Anspruch 1 oder 2 definierten Nukleinsäuresequenz in eine Wirtszelle umfasst.

4. Ein Verfahren nach Anspruch 3, dadurch gekennzeichnet, dass die Wirtszelle ein Bakterium ist.

5. Ein Verfahren zur Herstellung eines rekombinanten Virus, dadurch gekennzeichnet, dass die in Anspruch 1 oder 2 definierte Nukleinsäuresequenz in einen Virus einverleibt wird.

6. Ein Verfahren nach Anspruch 3 oder 4, welches das Exprimierenlassen der Nukleinsäuresequenz in eine Kultur und das Gewinnen des Polypeptids aus der Kultur umfasst.

7. Ein Verfahren zur Herstellung eines Impistoffes zum Schutz von Tieren gegen SchweinepestvirusInfektion, umfassend, im Anschluss an das Verfahren nach Anspruch 6, die Verarbeitung des Polypeptid-Expressionsproduktes zu einem pharmazeutischen Präparat mit immunisierender Wirkung.

8. Ein Verfahren zur Herstellung eines Impistoffes zum Schutz von Tieren gegen Schweinepestvirus-Infektion, umfassend die Verarbeitung einer Wirtszelle, die fähig ist, die in Anspruch 1 oder 2 definierte Nukleinsäuresequenz zu exprimieren, oder eines rekombinanten Virus, welcher die in Anspruch 1 oder 2 definierte Nukleinsäuresequenz umfasst, zu einem pharmazeutischen Präparat mit immunisierender Wirkung.

Revendications

Revendications pour les Etats contractants suivants : BE, CH, DE, DK, FR, GB, IT, LI, NL, SE

1. Séquence d'acide nucléique correspondant à un fragment du génome du virus de la peste porcine, et codant pour un polypeptide du virus de la peste porcine ou un fragment antigénique de celui-ci, susceptible d'induire des anticorps de neutralisation de virus, lequel polypeptide comprend la séquence d'acides aminés localisée au sein de la position des acides aminés 689-1067 représentée à la fig. 2 ou est un équivalent fonctionnel de celle-ci.

2. Séquence d'acide nucléique selon la revendication 1, caractérisée en ce qu'elle comprend soit la séquence nucléotidique 2428-3564 représentée à la fig. 2 ou un fragment de celle-ci, soit une séquence nucléotidique s'hybridant dans des conditions stringentes à ladite séquence nucléotidique représentée à la fig. 2.

3. Molécule d'acide nucléique recombinant comprenant une molécule d'acide nucléique vecteur et une séquence d'acide nucléique selon la revendication 1 ou la revendication 2.

4. Cellule hôte susceptible de produire un polypeptide du virus de la peste porcine comprenant une molécule d'acide nucléique recombinant selon la revendication 3.

5. Cellule hôte selon la revendication 4, caractérisée en ce que la cellule hôte est une bactérie.

6. Virus recombinant contenant une molécule d'acide nucléique recombinant selon la revendication 3.

7. Procédé de production d'un polypeptide du virus de la peste porcine, lequel polypeptide correspond à une portion de la polyprotéine du précurseur du virus de la pest porcine, susceptible d'induire des anticorps de neutralisation de virus, caractérisé en ce qu'une cellule hôte selon la revendication 4 ou 5, ou un virus recombinant selon la revendication 6 est propagé en culture dans des conditions t lles qu le polypeptide est exprimé, puis l polypeptide est isolé de la culture.

8. Vaccin destiné à protéger les animaux contre l'infection par le virus de la peste porcine, caractérisé en ce qu'il comprend un polypeptide du virus de la peste porcine, lequel polypeptide correspond à une portion de la polyprotéine précurseur du virus de la peste porcine, susceptible d'induire des anticorps de neutralisation de virus, lequel polypeptide comprend la séquence d'acides aminés localisée au sein de la position des acides aminés 689-1067 représentée à la fig. 2 *sous forme non dénaturée et non réduite*, ou est un fragment antigénique ou un équivalent fonctionnel de celle-ci.

9. Vaccin destiné à protéger les animaux contre l'infection par le virus de la peste porcine, caractérisé en ce qu'il comprend un polypeptide du virus de la peste porcine tel qu'on peut l'obtenir par l'expression dans une cellule hôte recombinante d'une séquence d'acide nucléique correspondant à un fragment du génome du virus de la peste porcine, et codant pour un polypeptide du virus de la peste porcine ou un fragment antigénique de celui-ci, susceptible d'induire des anticorps de neutralisation de virus, lequel polypeptide comprend la séquence d'acides aminés localisée au sein de la position des acides aminés 689-1067 représentée à la fig. 2 ou est un équivalent fonctionnel de celle-ci.

10. Vaccin selon la revendication 9, caractérisé en ce que ladite séquence d'acide nucléique comprend soit la séquence nucléotidique 2428-3564 représentée à la fig. 2 ou un fragment de celle-ci, soit une séquence nucléotidique s'hybridant dans des conditions stringentes à ladite séquence nucléotidique représentée à la fig. 2.

11. Vaccin destiné à protéger les animaux contre l'infection par le virus de la peste porcine, caractérisé en ce qu'il comprend une cellule hôte selon les revendications 4-5 ou un virus recombinant selon la revendication 6.

12. Procédé de préparation d'un vaccin du virus de la peste porcine, caractérisé en ce qu'un polypeptide tel que défini dans les revendications 8-10 est formé en une préparation pharmaceutique à activité immunisante.

13. Procédé de préparation d'un vaccin du virus de la peste porcine, caractérisé en ce que la cellule hôte selon les revendications 4-5 ou un virus recombinant selon la revendication 6 est propagé en culture, puis la cellule hôte ou le virus recombinant est récolté et formé en une préparation pharmaceutique à activité immunisante.

Revendications pour les Etats contractants suivants : ES, GR

1. Un procédé de préparation d'une molécule d'acide nucléique recombinante qui comprend l'incorporation d'une séquence nucléotidique correspondant à un fragment du génome du virus de la peste porcine, et codant pour un polypeptide du virus de la peste porcine ou un fragment antigénique de ce dernier, capable d'induire des anticorps neutralisants le virus, lequel polypeptide comprend la séquence d'acides aminés localisée entre les positions d'acide aminé 689-1067 décrite à la figure 2 ou correspond à un équivalent fonctionnel de ce dernier, dans une molécule d'acide nucléique vecteur.

2. Un procédé selon la revendication 1 caractérisé en ce que ladite séquence nucléotidique comprend soit la séquence nucléotidique 2428-3564 décrite à la figure 2 soit un fragment de cette dernière soit une séquence nucléotidique qui s'hybride dans des conditions drastiques à ladite séquence nucléotidique décrite à la figure 2.

3. Un procédé qui comprend l'expression dans une cellule hôte de la séquence d'acides nucléiques définie dans la revendication 1 ou 2.

4. Un procédé selon la revendication 3 caractérisée en ce que la cellule hôte est une bactérie.

5. Un procédé de préparation d'un virus recombinant caractérisé en ce que la séquence d'acides nucléiques définie dans la revendication 1 ou 2 est incorporée dans un virus.

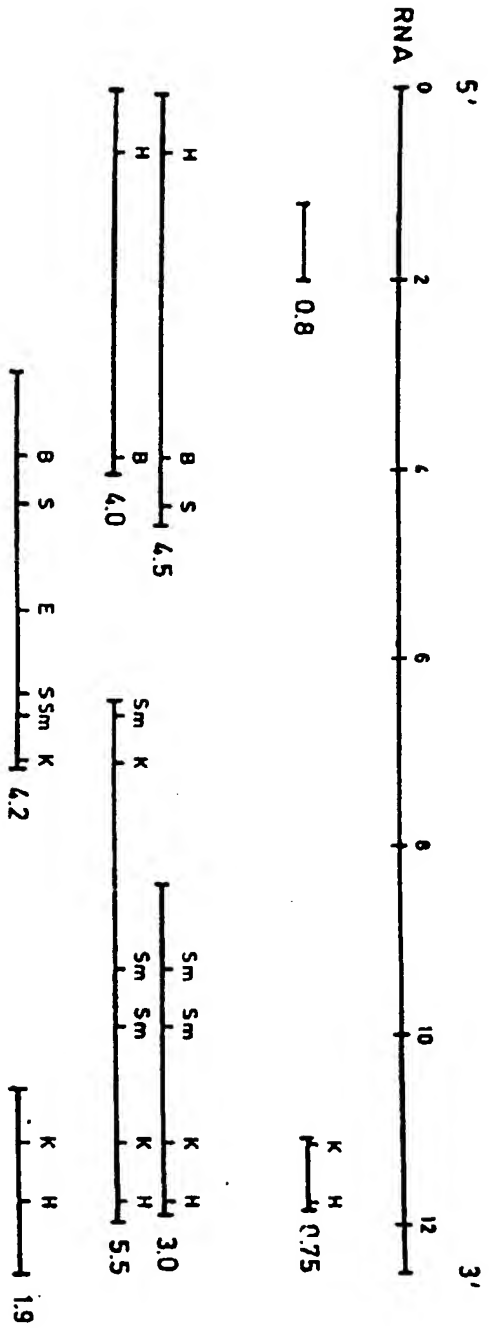
6. Un procédé selon la revendication 3 ou 4 qui comprend l'expression de la séquence d'acides nucléiques dans une

culture et la récupération du polypeptide à partir de la culture.

7. Un procédé d préparation d'un vaccin pour la protection des animaux contr une inf ction par le virus de la peste porcine comprenant. en suivant le procédé de la revendication 6, la transformation du produit d'expression du polypeptide en une préparation pharmaceutique présentant une activité immunisante.

8. Un procédé de préparation d'un vaccin pour la protection des animaux contre une infection par le virus de la peste porcine comprenant la transformation d'une cellule hôte capable d'exprimer la séquence d'acides nucléiques définie dans les revendications 1 ou 2 ou d'un virus recombinant comprenant la séquence d'acides nucléiques définie dans les revendications 1 ou 2, en une composition pharmaceutique présentant une activité immunisante.

Figure 1.


5'
RNA
0
2
4
6
8
10
12
3'
0.8
H
H
B
B
4.0
B S
S
4.5
B S E S Sm K
4.2
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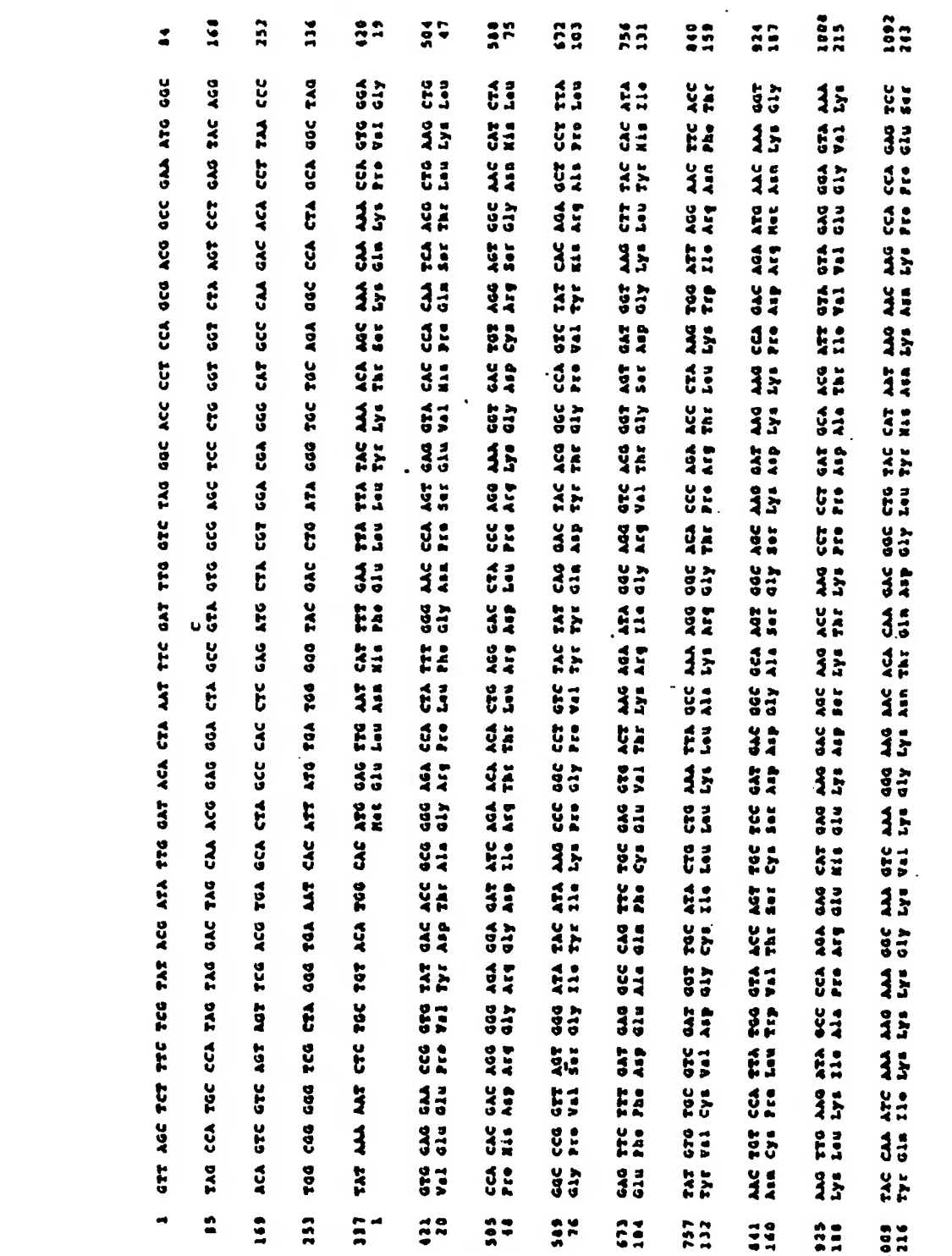
5.5
K H
4 0.75
K H
1.9

Figure 2.



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 244  Arg Lys Lys Leu Glu Lys Ala Leu Leu Ala Trp Ala Val Ile Thr Ile Leu Leu Tyr Gln Pro Val Ala Ala Glu Asn Ile Thr   271

1177  CAA TGG AAC CTG AGT GAC AAC GGC ACT AAT GGT ATT CAG CGA GCC ATG TAT CTT AGA GGG GTT AAC AGG AGC TTA CAT GGG ATC   1260
 272  Gln Trp Asn Leu Ser Asp Asn Gly Thr Asn Gly Ile Gln Arg Ala Met Tyr Leu Arg Gly Val Asn Arg Ser Leu His Gly Ile   299

1261  TGG CCC GAG AAA ATA TGC AAG GGG GTC CCC ACT CAT CTG GCC ACT GAC ACG GAA CTG AAA GAG ATA CGC GGG ATG ATG GAT GCC   1344
 300  Trp Pro Glu Lys Ile Cys Lys Gly Val Pro Thr His Leu Ala Thr Asp Thr Glu Leu Lys Glu Ile Arg Gly Met Met Asp Ala   327

1345  AGC GAG AGG ACA AAC TAT ACG TGC TGT AGG TTA CAA AGA CAT GAA TGG AAC AAA CAT GGA TGG TGT AAC TGG TAC AAC ATA GAC   1428
 328  Ser Glu Arg Thr Asn Tyr Thr Cys Cys Arg Leu Gln Arg His Glu Trp Asn Lys His Gly Trp Cys Asn Trp Tyr Asn Ile Asp   355

1429  CCT TGG ATT CAG TTA ATG AAC AGG ACC CAA ACA AAT TTG ACA GAA GGC CCT CCA GAT AAG GAG TGT GCC GTG ACC TGC AGG TAT   1512
 356  Pro Trp Ile Gln Leu Met Asn Arg Thr Gln Thr Asn Leu Thr Glu Gly Pro Pro Asp Lys Glu Cys Ala Val Thr Cys Arg Tyr   383

                G
1513  GAC AAA AAT ACC GAT GTC AAC GTG GTC ACC CAG GCC AGG AAT AGG CCA ACT ACT CTG ACT GGC TGC AAG AAA GGG AAA AAC TTT   1596
 384  Asp Lys Asn Thr Asp Val Asn Val Val Thr Gln Ala Arg Asn Arg Pro Thr Thr Leu Thr Gly Cys Lys Lys Gly Lys Asn Phe   411
           Ala

1597  TCA TTC GCA GGC ACA GTC ATA GAG GGC CCG TGC AAT TTC AAC GTT TCC GTG GAG GAC ATC TTA TAC GGA GAC CAT GAG TGT GGC   1680
 412  Ser Phe Ala Gly Thr Val Ile Glu Gly Pro Cys Asn Phe Asn Val Ser Val Glu Asp Ile Leu Tyr Gly Asp His Glu Cys Gly   439

1681  AGT CTG CTC CAG GAC ACG GCT CTG TAC CTA TTG GAT GGA ATG ACC AAC ACT ATA GAG AAT GCC AGG CAA GGT GCG GCG CGG GTG   1764
 440  Ser Leu Leu Gln Asp Thr Ala Leu Tyr Leu Leu Asp Gly Met Thr Asn Thr Ile Glu Asn Ala Arg Gln Gly Ala Ala Arg Val   467

1765  ACA TCT TGG CTT GGG AGG CAG CTC AGT ACC GCA GGG AAG AAG CTA GAG AGG AGA AGC AAA ACC TGG TTT GGT GCC TAT GCC CTG   1848
 468  Thr Ser Trp Leu Gly Arg Gln Leu Ser Thr Ala Gly Lys Lys Leu Glu Arg Arg Ser Lys Thr Trp Phe Gly Ala Tyr Ala Leu   495

1849  TCA CCT TAC TGC AAT GTG ACT AGA AAA ATA GGG TAC ATA TGG TAT ACA AAC AAC TGC ACC CCG GCA TGC CTC CCT AAG AAC ACA   1932
 496  Ser Pro Tyr Cys Asn Val Thr Arg Lys Ile Gly Tyr Ile Trp Tyr Thr Asn Asn Cys Thr Pro Ala Cys Leu Pro Lys Asn Thr   523

1933  AAA ATA ATA GGC CCT GGA AAG TTT GAC ACC AAT GCG GAA GAC GGG AAG ATC CTT CAT GAA ATG GGG GGC CAC CTA TCA GAA TTT   2016
 524  Lys Ile Ile Gly Pro Gly Lys Phe Asp Thr Asn Ala Glu Asp Gly Lys Ile Leu His Glu Met Gly Gly His Leu Ser Glu Phe   551

2017  TTG TTG CTT TCT CTA GTT ATC CTG TCT GAC TTT GCC CCC GAG ACA GCT AGC ACG CTA TAC CTA ATT TTA CAC TAT GCA ATC CCC   2100
 552  Leu Leu Leu Ser Leu Val Ile Leu Ser Asp Phe Ala Pro Glu Thr Ala Ser Thr Leu Tyr Leu Ile Leu His Tyr Ala Ile Pro   579
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2101  CAG TCC CAC GAA GAA CCT GAA GGT TGT GAT ACG AAC CAA CTT AAC CTA ACA GTG AAA CTT AGG ACA GAA GAC GTA GTG CCA TCA   2184
 580  Gln Ser His Glu Glu Pro Glu Gly Cys Asp Thr Asn Gln Leu Asn Leu Thr Val Lys Leu Arg Thr Glu Asp Val Val Pro Ser    607

2185  TCA GTT TGG AAT ATT GGC AAA TAT GTT TGT GTT AGA CCA GAC TGG TGG CCG TAT GAA ACT AAA GTG GCT CTG CTG TTT GAA GAG   2268
 608  Ser Val Trp Asn Ile Gly Lys Tyr Val Cys Val Arg Pro Asp Trp Trp Pro Tyr Glu Thr Lys Val Ala Leu Leu Phe Glu Glu    635

2269  GCA GGA CAG GTT ATA AAG CTA GTC CTA CGG GCA CTG AGG GAT TTA ACT AGG GTC TGG AAC AGC GCA TCA ACT ACT GCG TTT CTC   2352
 636  Ala Gly Gln Val Ile Lys Leu Val Leu Arg Ala Leu Arg Asp Leu Thr Arg Val Trp Asn Ser Ala Ser Thr Thr Ala Phe Leu    663

2353  ATT TGC TTG ATA AAA GTA TTG AGA GGA CAG GTT GTG CAA GGT ATA ATA TGG CTG CTG CTG GTG ACC GGG GCA CAA GGG CGG CTA   2436
 664  Ile Cys Leu Ile Lys Val Leu Arg Gly Gln Val Val Gln Gly Ile Ile Trp Leu Leu Leu Val Thr Gly Ala Gln Gly Arg Leu    691

2437  GCC TGT AAG GAA GAC TAC AGG TAT GCG ATC TCG TCA ACC AAT GAG ATA GGG CTG CTG GGC GCT GAA GGT CTC ACC ACT ACC TGG   2520
 692  Ala Cys Lys Glu Asp Tyr Arg Tyr Ala Ile Ser Ser Thr Asn Glu Ile Gly Leu Leu Gly Ala Glu Gly Leu Thr Thr Thr Trp    719

2521  AAA GAA TAC AGC CAC GGT TTG CAG CTG GAC GAC GGA ACC GTT AAG GCC GTC TGC ACT GCA GGG TCC TTT AAA GTC ACA GCA CTT   2604
 720  Lys Glu Tyr Ser His Gly Leu Gln Leu Asp Asp Gly Thr Val Lys Ala Val Cys Thr Ala Gly Ser Phe Lys Val Thr Ala Leu    747

2605  AAC GTG GTT AGT AGG AGG TAT CTA GCA TCA TTG CAC AAG AGG GCT CTA CCC ACC TCA GTG ACA TTT GAG CTC CTA TTT GAC GGG   2688
 748  Asn Val Val Ser Arg Arg Tyr Leu Ala Ser Leu His Lys Arg Ala Leu Pro Thr Ser Val Thr Phe Glu Leu Leu Phe Asp Gly    775

2689  ACC AAC CCA GCA ATC GAG GAG ATG GAT GAT GAC TTC GGA TTT GGG CTG TGC CCA TTT GAC ACG AGT CCT GTG ATC AAA GGG AAG   2772
 776  Thr Asn Pro Ala Ile Glu Glu Met Asp Asp Asp Phe Gly Phe Gly Leu Cys Pro Phe Asp Thr Ser Pro Val Ile Lys Gly Lys    803

2773  TAC AAC ACC ACT TTG TTA AAC GGC AGT GCT TTC TAT CTA GTC TGC CCA ATA GGA TGG ACT GGT GTC GTA GAG TGC ACA GCA GTG   2856
 804  Tyr Asn Thr Thr Leu Leu Asn Gly Ser Ala Phe Tyr Leu Val Cys Pro Ile Gly Trp Thr Gly Val Val Glu Cys Thr Ala Val    831

2857  AGC CCC ACA ACC TTG AGA ACA GAA GTG GTG AAA ACC TTC AGG AGA GAT AAG CCT TTT CCA CAT AGA GTA GAC TGT GTG ACC ACC   2940
 832  Ser Pro Thr Thr Leu Arg Thr Glu Val Val Lys Thr Phe Arg Arg Asp Lys Pro Phe Pro His Arg Val Asp Cys Val Thr Thr    859

2941  ATA GTA GAA AAA GAA GAC CTA TTC CAT TGC AAG TTG GGG GGT AAT TGG ACA TGT GTA AAA GGC GAC CCA GTG ACT TAT AAG GGG   3024
 860  Ile Val Glu Lys Glu Asp Leu Phe His Cys Lys Leu Gly Gly Asn Trp Thr Cys Val Lys Gly Asp Pro Val Thr Tyr Lys Gly    887

3025  GGG CAA GTA AAG CAG TGC AGG TGG TGT GGT TTC GAG TTT AAA GAG CCC TAC GGG CTC CCA CAC TAC CCT ATA GGC AAG TGC ATC   3108
 888  Gly Gln Val Lys Gln Cys Arg Trp Cys Gly Phe Glu Phe Lys Glu Pro Tyr Gly Leu Pro His Tyr Pro Ile Gly Lys Cys Ile    915
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3109  CTA ACA AAT GAG ACA GGT TAC AGG GTA GTA GAT TCC ACA GAC TGC AAC AGA GAT GGC GTC GTT ATT AGC ACT GAA GGG GAA CAT   3192
 916  Leu Thr Asn Glu Thr Gly Tyr Arg Val Val Asp Ser Thr Asp Cys Asn Arg Asp Gly Val Val Ile Ser Thr Glu Gly Glu His   943

3193  GAG TGC TTG ATT GGC AAC ACT ACC GTC AAG GTG CAT GCA CTG GAT GAA AGA TTG GGC CCT ATG CCG TGC AGA CCC AAA GAA ATC   3276
 944  Glu Cys Leu Ile Gly Asn Thr Thr Val Lys Val His Ala Leu Asp Glu Arg Leu Gly Pro Met Pro Cys Arg Pro Lys Glu Ile   971

3277  GTC TCT AGT GAG GGA CCT GTG AGG AAA ACT TCT TGT ACA TTC AAC TAC ACA AAG ACT CTA AGA AAC AAA TAC TAT GAG CCC AGA   3360
 972  Val Ser Ser Glu Gly Pro Val Arg Lys Thr Ser Cys Thr Phe Asn Tyr Thr Lys Thr Leu Arg Asn Lys Tyr Ty- Glu Pro Arg   999

3361  GAC AGT TAC TTC CAG CAA TAT ATG CTC AAG GGC GAG TAT CAA TAC TGG TTT AAT CTG GAC GTG ACC GAC CAC CAC ACA GAC TAC   3444
1000  Asp Ser Tyr Phe Gln Gln Tyr Met Leu Lys Gly Glu Tyr Gln Tyr Trp Phe Asn Leu Asp Val Thr Asp His His Thr Asp Tyr   1027

3445  TTT GCC GAG TTT GTT GTC TTG GTA GTA GTA GCA CTG TTA GGA GGA AGG TAC GTT CTG TGG CTA ATA GTG ACC TAC ATA ATT CTA   3528
1028  Phe Ala Glu Phe Val Val Leu Val Val Val Ala Leu Leu Gly Gly Arg Tyr Val Leu Trp Leu Ile Val Thr Tyr Ile Ile Leu   1055

3529  ACA GAG CAG CTC GCT GCT GGT CTA CAG CTA GGC CAG GGT GAG GTG GTA TTG ATA GGG AAC CTA ATT ACC CAC ACG GAC AAT GAG   3612
1056  Thr Glu Gln Leu Ala Ala Gly Leu Gln Leu Gly Gln Gly Glu Val Val Leu Ile Gly Asn Leu Ile Thr His Thr Asp Asn Glu   1083

3613  GTG GTG GTG TAC TTC CTA CTG CTC TAC TTA GTA ATA AGA GAT GAG CCC ATA AAG AAA TGG ATA CTA CTG CTG TTT CAT GCA ATG   3696
1084  Val Val Val Tyr Phe Leu Leu Leu Tyr Leu Val Ile Arg Asp Glu Pro Ile Lys Lys Trp Ile Leu Leu Leu Phe His Ala Met   1111

3697  ACT AAC AAT CCA GTC AAG ACC ATA ACA GTA GCA TTG CTA ATG ATC AGT GGG GTT GCC AAG GGT GGT AAG ATA GAT GGT GGC TGG   3780
1112  Thr Asn Asn Pro Val Lys Thr Ile Thr Val Ala Leu Leu Met Ile Ser Gly Val Ala Lys Gly Gly Lys Ile Asp Gly Gly Trp   1139

3781  CAG AGA CAA CCG GTG ACC AGT TTT GAC ATC CAA CTC GCA CTG GCA GTC GTA GTA GTC GTT GTG ATG TTG CTG GCA AAG AGA GAC   3864
1140  Gln Arg Gln Pro Val Thr Ser Phe Asp Ile Gln Leu Ala Leu Ala Val Val Val Val Val Val Met Leu Leu Ala Lys Arg Asp   1167

3865  CCG ACT ACT TTC CCT TTG GTA ATC ACA GTG GCA ACC CTG AGA ACG GCC AAG ATA ACC AAC GGT TTT AGC ACA GAT CTA GTC ATA   3948
1168  Pro Thr Thr Phe Pro Leu Val Ile Thr Val Ala Thr Leu Arg Thr Ala Lys Ile Thr Asn Gly Phe Ser Thr Asp Leu Val Ile   1195

3949  GCC ACA GTG TCG GCA GCT TTG TTA ACT TGG ACC TAT ATC AGC GAC TAC TAC AAA TAC AAG ACT TGG CTA CAG TAC CTC GTC AGC   4032
1196  Ala Thr Val Ser Ala Ala Leu Leu Thr Trp Thr Tyr Ile Ser Asp Tyr Tyr Lys Tyr Lys Thr Trp Leu Gln Tyr Leu Val Ser   1223

4033  ACG GTG ACT GGA ATC TTC CTG ATA AGG GTG CTG AAG GGA ATA GGC GAA TTG GAT CTG CAC GCC CCA ACC TTG CCG TCT CAC AGA   4116
1224  Thr Val Thr Gly Ile Phe Leu Ile Arg Val Leu Lys Gly Ile Gly Glu Leu Asp Leu His Ala Pro Thr Leu Pro Ser His Arg   1251
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4117  CCC CTC TTT TAC ATC CTT GTA TAC CTT ATT TCC ACT GCC GTG GTA ACT AGA TGG AAT CTG GAC GTA GCC GGA TTG TTG CTG CAG   4200
1252  Pro Leu Phe Tyr Ile Leu Val Tyr Leu Ile Ser Thr Ala Val Val Thr Arg Trp Asn Leu Asp Val Ala Gly Leu Leu Leu Gln   1279

4201  TGC GTC CCA ACT CTT TTA ATG GTT TTT ACG ATG TGG GCA GAC ATT CTC ACC CTA ATT CTC ATA CTA CCT ACT TAT GAG TTA ACA   4284
1280  Cys Val Pro Thr Leu Leu Met Val Phe Thr Met Trp Ala Asp Ile Leu Thr Leu Ile Leu Ile Leu Pro Thr Tyr Glu Leu Thr   1307

4285  AAG TTA TAC TAC CTT AAG GAA GTG AAG ATT GGG GCA GAA AGA GGT TGG CTG TGG AAA ACT AAC TAT AAG AGG GTA AAC GAC ATC   4368
1308  Lys Leu Tyr Tyr Leu Lys Glu Val Lys Ile Gly Ala Glu Arg Gly Trp Leu Trp Lys Thr Asn Tyr Lys Arg Val Asn Asp Ile   1335

4369  TAC GAG GTC GAC CAA ACT AGC GAA GGG GTT TAC CTT TTC CCT TCT AAA CAG AGG ACG AGC GCT ATA ACT AGT ACC ATG TTG CCA   4452
1336  Tyr Glu Val Asp Gln Thr Ser Glu Gly Val Tyr Leu Phe Pro Ser Lys Gln Arg Thr Ser Ala Ile Thr Ser Thr Met Leu Pro   1363

4453  TTA ATC AAA GCC ATA CTC ATT AGC TGC ATC AGC AAC AAG TGG CAA CTC ATA TAC TTA CTG TAC TTG ATA TTT GAA GTG TCT TAC   4536
1364  Leu Ile Lys Ala Ile Leu Ile Ser Cys Ile Ser Asn Lys Trp Gln Leu Ile Tyr Leu Leu Tyr Leu Ile Phe Glu Val Ser Tyr   1391

4537  TAC CTC CAC AAG AAA GTT ATA GAT GAA ATA GCT GGT GGG ACC AAC TTC GTT TCA AGG CTC GTG GCG GCT TTG ATT GAA GTC AAT   4620
1392  Tyr Leu His Lys Lys Val Ile Asp Glu Ile Ala Gly Gly Thr Asn Phe Val Ser Arg Leu Val Ala Ala Leu Ile Glu Val Asn   1419

4621  TGG GCC TTC GAC AAT GAA GAA GTC AAA GGC TTA AAG AAG TTC TTC TTG CTG TCT AGT AGG GTC AAA GAG TTG ATC ATC AAA CAC   4704
1420  Trp Ala Phe Asp Asn Glu Glu Val Lys Gly Leu Lys Lys Phe Phe Leu Leu Ser Ser Arg Val Lys Glu Leu Ile Ile Lys His   1447

4705  AAA GTG AGG AAT GAA GTA GTG GTC CGC TGG TTT GGA GAT GAA GAG ATT TAT GGG ATG CCA AAG CTG ATC GGC TTA GTT AAG GCA   4788
1448  Lys Val Arg Asn Glu Val Val Val Arg Trp Phe Gly Asp Glu Glu Ile Tyr Gly Met Pro Lys Leu Ile Gly Leu Val Lys Ala   1475

4789  GCA ACA CTA AGT AGA AAC AAA CAC TGT ATG TTG TGT ACC GTC TGT GAG GAC AGA GAT TGG AGA GGG GAA ACT TGC CCT AAA TGT   4872
1476  Ala Thr Leu Ser Arg Asn Lys His Cys Met Leu Cys Thr Val Cys Glu Asp Arg Asp Trp Arg Gly Glu Thr Cys Pro Lys Cys   1503

4873  GGG CGT TTT GGA CCA CCA GTG GTC TGC GGT ATG ACC CTA GCC GAT TTC GAA GAA AAA CAC TAT AAA AGG ATT TTC ATT AGA GAG   4956
1504  Gly Arg Phe Gly Pro Pro Val Val Cys Gly Met Thr Leu Ala Asp Phe Glu Glu Lys His Tyr Lys Arg Ile Phe Ile Arg Glu   1531

4957  GAC CAA TCA GGC GGG CCA CTT AGG GAG GAG CAT GCA GGG TAC TTG CAG TAC AAA GCC AGG GGT CAA CTG TTT TTG AGG AAC CTC   5040
1532  Asp Gln Ser Gly Gly Pro Leu Arg Glu Glu His Ala Gly Tyr Leu Gln Tyr Lys Ala Arg Gly Gln Leu Phe Leu Arg Asn Leu   1559

5041  CCA GTG TTA GCT ACA AAA GTC AAG ATG CTC CTG GTT GGT AAC CTC GGG ACA GAG ATT GGG GAT CTG GAA CAC CTT GGC TGG GTG   5124
1560  Pro Val Leu Ala Thr Lys Val Lys Met Leu Leu Val Gly Asn Leu Gly Thr Glu Ile Gly Asp Leu Glu His Leu Gly Trp Val   1587
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5125  CTT AGA GGG CCA GCT GTT TGC AAG AAG GTT ACT GAA CAC GAA AGA TGC ACC ACG TCT ATA ATG GAT AAG TTG ACT GCT TTC TTT   5208
1588  Leu Arg Gly Pro Ala Val Cys Lys Lys Val Thr Glu His Glu Arg Cys Thr Thr Ser Ile Met Asp Lys Leu Thr Ala Phe Phe   1615

5209  GGA GTA ATG CCA AGG GGC ACT ACT CCC AGA GCT CCC GTA AGA TTC CCT ACC TCC CTC CTA AAG ATA AGA AGA GGG CTG GAG ACT   5292
1616  Gly Val Met Pro Arg Gly Thr Thr Pro Arg Ala Pro Val Arg Phe Pro Thr Ser Leu Leu Lys Ile Arg Arg Gly Leu Glu Thr   1643

5293  GGT TGG GCT TAC ACA CAC CAA GGT GGC ATC AGC TCA GTA GAC CAT GTC ACT TGT GGG AAA GAC TTA CTG GTG TGT GAC ACC ATG   5376
1644  Gly Trp Ala Tyr Thr His Gln Gly Gly Ile Ser Ser Val Asp His Val Thr Cys Gly Lys Asp Leu Leu Val Cys Asp Thr Met   1671

5377  GGT CGG ACA AGG GTT GTT TGC CAG TCA AAT AAT AAG ATG ACC GAC GAG TCC GAA TAC GGA GTC AAA ACT GAC TCC GGG TGC CCA   5460
1672  Gly Arg Thr Arg Val Val Cys Gln Ser Asn Asn Lys Met Thr Asp Glu Ser Glu Tyr Gly Val Lys Thr Asp Ser Gly Cys Pro   1699

5461  GAG GGA GCC AGG TGT TAC GTG TTT AAC CCG GAA GCA GTT AAC ATA TCA GGC ACT AAA GGA GCC ATG GTC CAC TTA CAG AAA ACG   5544
1700  Glu Gly Ala Arg Cys Tyr Val Phe Asn Pro Glu Ala Val Asn Ile Ser Gly Thr Lys Gly Ala Met Val His Leu Gln Lys Thr   1727

5545  GGT GGA GAA TTC ACC TGT GTG ACA GCA TCA GGA ACC CCG GCC TTC TTT GAC CTC AAG AAC CTT AAG GGC TGG TCA GGG CTA CCG   5628
1728  Gly Gly Glu Phe Thr Cys Val Thr Ala Ser Gly Thr Pro Ala Phe Phe Asp Leu Lys Asn Leu Lys Gly Trp Ser Gly Leu Pro   1755

5629  ATA TTT GAA GCA TCA AGT GGA AGG GTA GTC GGA AGG GTC AAG GTC GGG AAG AAC GAG GAT TCC AAA CCA ACC AAG CTC ATG AGT   5712
1756  Ile Phe Glu Ala Ser Ser Gly Arg Val Val Gly Arg Val Lys Val Gly Lys Asn Glu Asp Ser Lys Pro Thr Lys Leu Met Ser   1783

5713  GGG ATA CAA ACG GTT TCT AAA AGC GCC ACA GAC TTG ACG GAG ATG GTG AAG AAG ATA ACG ACC ATG AAC AGG GGA GAG TTC AGA   5796
1784  Gly Ile Gln Thr Val Ser Lys Ser Ala Thr Asp Leu Thr Glu Met Val Lys Lys Ile Thr Thr Met Asn Arg Gly Glu Phe Arg   1811

5797  CAA ATA ACC CTG GCC ACA GGT GCC GGA AAA ACT ACA GAG CTC CCT AGA TCA GTT ATA GAA GAG ATA GGG AGG CAT AAG AGG GTG   5880
1812  Gln Ile Thr Leu Ala Thr Gly Ala Gly Lys Thr Thr Glu Leu Pro Arg Ser Val Ile Glu Glu Ile Gly Arg His Lys Arg Val   1839

5881  TTG GTC TTA ATC CCC TTG AGG GCG GCA GCA GAA TCA GTA TAC CAA TAC ATG AGA CAG AAA CAT CCG AGT ATA GCA TTC AAT CTA   5964
1840  Leu Val Leu Ile Pro Leu Arg Ala Ala Ala Glu Ser Val Tyr Gln Tyr Met Arg Gln Lys His Pro Ser Ile Ala Phe Asn Leu   1867

5965  AGG ATA GGT GAG ATG AAG GAA GGT GAT ATG GCC ACG GGA ATA ACC TAT GCC TCT TAC GGT TAC TTT TGC CAG ATG TCA CAA CCC   6048
1868  Arg Ile Gly Glu Met Lys Glu Gly Asp Met Ala Thr Gly Ile Thr Tyr Ala Ser Tyr Gly Tyr Phe Cys Gln Met Ser Gln Pro   1895

6049  AAG CTG AGA GCC GCA ATG GTA GAA TAT TCC TTT ATA TTC CTA GAT GAG TAT CAT TGT GCT ACC CCA GAA CAA CTG GCA ATC ATG   6132
1896  Lys Leu Arg Ala Ala Met Val Glu Tyr Ser Phe Ile Phe Leu Asp Glu Tyr His Cys Ala Thr Pro Glu Gln Leu Ala Ile Met   1923
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6131  GGG AAG ATC CAC AGA TTC TCA GAA AAC CTG CGG GTG GTA GCT ATG ACA GCG ACA CCG GCA GGC ACA GTA ACA ACC ACT GGG CAG  6216
1924  Gly Lys Ile His Arg Phe Ser Glu Asn Leu Arg Val Val Ala Met Thr Ala Thr Pro Ala Gly Thr Val Thr Thr Thr Gly Gln  1951

6217  AAA CAC CCT ATA GAG GAA TTT ATA GCC CCG GAA GTG ATG AAA GGA GAA GAC TTG GGT TCT GAG TAC TTA GAT ATT GCC GGA CTG  6300
1952  Lys His Pro Ile Glu Glu Phe Ile Ala Pro Glu Val Met Lys Gly Glu Asp Leu Gly Ser Glu Tyr Leu Asp Ile Ala Gly Leu  1979

6301  AAG ATA CCA GTA GAG GAG ATG AAG AAT AAC ATG CTA GTT TTT GTG CCC ACC AGG AAC ATG GCG GTA GAG GCG GCA AAG AAA TTG  6384
1980  Lys Ile Pro Val Glu Glu Met Lys Asn Asn Met Leu Val Phe Val Pro Thr Arg Asn Met Ala Val Glu Ala Ala Lys Lys Leu  2007

6385  AAG GCC AAA GGA TAC AAC TCG GGC TAC TAC TAC AGC GGA GAG GAC CCA TCT AAC CTG AGG GTG GTG ACG TCG CAG TCC CCA TAC  6468
2008  Lys Ala Lys Gly Tyr Asn Ser Gly Tyr Tyr Tyr Ser Gly Glu Asp Pro Ser Asn Leu Arg Val Val Thr Ser Gln Ser Pro Tyr  2035

6469  GTG GTG GTA GCA ACC AAC GCA ATA GAA TCG GGC GTT ACC CTC CCG GAC CTG GAC GTG GTT GTC GAC ACG GGA CTC AAG TGT GAA  6552
2036  Val Val Val Ala Thr Asn Ala Ile Glu Ser Gly Val Thr Leu Pro Asp Leu Asp Val Val Val Asp Thr Gly Leu Lys Cys Glu  2063

6553  AAA AGA ATC CGA CTG TCA CCC AAG ATG CCT TTC ATA GTG ACG GGC CTG AAA AGA ATG GCC GTC ACT ATT GGG GAA CAA GCC CAG  6636
2064  Lys Arg Ile Arg Leu Ser Pro Lys Met Pro Phe Ile Val Thr Gly Leu Lys Arg Met Ala Val Thr Ile Gly Glu Gln Ala Gln  2091

6637  AGA AGA GGG AGG GTT GGA AGA GTG AAG CCC GGG AGA TAC TAC AGG AGT CAA GAA ACA CCT GTC GGC TCT AAA GAC TAC CAT TAT  6720
2092  Arg Arg Gly Arg Val Gly Arg Val Lys Pro Gly Arg Tyr Tyr Arg Ser Gln Glu Thr Pro Val Gly Ser Lys Asp Tyr His Tyr  2119

6721  GAC TTA TTG CAA GCC CAG AGG TAC GGC ATA GAA GAT GGG ATA AAT ATC ACC AAA TCC TTC AGA GAG ATG AAC TAC GAC TGG AGC  6804
2120  Asp Leu Leu Gln Ala Gln Arg Tyr Gly Ile Glu Asp Gly Ile Asn Ile Thr Lys Ser Phe Arg Glu Met Asn Tyr Asp Trp Ser  2147

6805  CTT TAT GAG GAA GAT AGC CTG ATG ATC ACA CAA CTG GAA ATC CTC AAC AAC CTG TTG ATA TCA GAA GAG CTG CCG ATG GCA GTA  6888
2148  Leu Tyr Glu Glu Asp Ser Leu Met Ile Thr Gln Leu Glu Ile Leu Asn Asn Leu Leu Ile Ser Glu Glu Leu Pro Met Ala Val  2175

6889  AAA AAT ATA ATG GCC AGG ACC GAC CAC CCA GAA CCA ATT CAA CTC GCG TAT AAC AGC TAC GAG ACA CAG GTG CCG GTA TTA TTC  6972
2176  Lys Asn Ile Met Ala Arg Thr Asp His Pro Glu Pro Ile Gln Leu Ala Tyr Asn Ser Tyr Glu Thr Gln Val Pro Val Leu Phe  2203

6973  CCA AAA ATA AGA AAT GGA GAG GTG ACT GAT ACT TAC GAT AAT TAC ACC TTC CTC AAT GCA AGA AAA TTG GGA GAT GAC GTA CCC  7056
2204  Pro Lys Ile Arg Asn Gly Glu Val Thr Asp Thr Tyr Asp Asn Tyr Thr Phe Leu Asn Ala Arg Lys Leu Gly Asp Asp Val Pro  2231

7057  CCC TAC GTG TAT GCT ACA GAG GAT GAG GAC TTG GCA GTG GAA CTG TTG GGC CTA GAT TGG CCG GAC CCA GGA AAC CAA GGC ACC  7140
2232  Pro Tyr Val Tyr Ala Thr Glu Asp Glu Asp Leu Ala Val Glu Leu Leu Gly Leu Asp Trp Pro Asp Pro Gly Asn Gln Gly Thr  2259
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7141  GTG GAA GCT GGC AGA GCA CTA AAA CAG GTG GTT GGT CTA TCA ACA GCA GAG AAC GCC CTG CTA GTC GCC CTG TTC GGC TAC GTG   7224
2260  Val Glu Ala Gly Arg Ala Leu Lys Gln Val Val Gly Leu Ser Thr Ala Glu Asn Ala Leu Leu Val Ala Leu Phe Gly Tyr Val   2287

7225  GGG TAC CAG GCG CTT TCA AAG AGA CAT ATA CCA GTG GTC ACA GAT ATA TAT TCA GTA GAA GAT CAC AGG CTA GAG GAC ACT ACG   7308
2288  Gly Tyr Gln Ala Leu Ser Lys Arg His Ile Pro Val Val Thr Asp Ile Tyr Ser Val Glu Asp His Arg Leu Glu Asp Thr Thr   2315

7309  CAC CTA CAG TAT GCT CCG AAT GCC ATC AAG ACG GAG GGG AAG GAA ACT GAA TTG AAG GAG CTG GCT CAG GGG GAT GTG CAG AGA   7392
2316  His Leu Gln Tyr Ala Pro Asn Ala Ile Lys Thr Glu Gly Lys Glu Thr Glu Leu Lys Glu Leu Ala Gln Gly Asp Val Gln Arg   2343

7393  TGT GTG GAA GCA GTG ACC AAT TAT GCG AGA GAG GGC ATC CAA TTC ATG AAG TCG CAG GCA CTG AAA GTG AGA GAA ACC CCT ACC   7476
2344  Cys Val Glu Ala Val Thr Asn Tyr Ala Arg Glu Gly Ile Gln Phe Met Lys Ser Gln Ala Leu Lys Val Arg Glu Thr Pro Thr   2371

7477  TAT AAA GAG ACA ATG AAC ACC GTG GCA GAT TAT GTG AAA AAG TTT ATT GAG GCA CTG ACG GAT AGC AAG GAA GAC ATC ATT AAA   7560
2372  Tyr Lys Glu Thr Met Asn Thr Val Ala Asp Tyr Val Lys Lys Phe Ile Glu Ala Leu Thr Asp Ser Lys Glu Asp Ile Ile Lys   2399

7561  TAT GGG CTG TGG GGG GCA CAT ACG GCA TTG TAT AAG AGC ATT GGT GCC AGG CTT GGT CAC GAA ACC GCG TTC GCA ACT CTA GTT   7644
2400  Tyr Gly Leu Trp Gly Ala His Thr Ala Leu Tyr Lys Ser Ile Gly Ala Arg Leu Gly His Glu Thr Ala Phe Ala Thr Leu Val   2427

7645  GTG AAG TGG TTG GCA TTT GGG GGG GAG TCA ATA TCA GAC CAC ATA AAG CAA GCG GCC ACA GAC TTG GTC GTT TAT TAC ATT ATT   7728
2428  Val Lys Trp Leu Ala Phe Gly Gly Glu Ser Ile Ser Asp His Ile Lys Gln Ala Ala Thr Asp Leu Val Val Tyr Tyr Ile Ile   2455

7729  AAC AGA CCT CAA TTC CCA GGA GAC ACA GAA ACA CAA CAA GAA GGG AGA AAA TTT GTT GCC AGC CTG CTA GTC TCA GCT CTA GCG   7812
2456  Asn Arg Pro Gln Phe Pro Gly Asp Thr Glu Thr Gln Gln Glu Gly Arg Lys Phe Val Ala Ser Leu Leu Val Ser Ala Leu Ala   2483

7813  ACT TAT ACA TAC AAG AGC TGG AAC TAC AAT AAT CTG TCC AAA ATA GTT GAA CCG GCT TTG GCT ACC CTG CCC TAT GCC GCT AAA   7896
2484  Thr Tyr Thr Tyr Lys Ser Trp Asn Tyr Asn Asn Leu Ser Lys Ile Val Glu Pro Ala Leu Ala Thr Leu Pro Tyr Ala Ala Lys   2511

7897  GCC CTC AAG CTA TTT GCT CCT ACC CGA CTG GAG AGC GTT GTC ATA CTG AGC ACT GCA ATC TAC AAA ACA TAC CTA TCA ATA AGG   7980
2512  Ala Leu Lys Leu Phe Ala Pro Thr Arg Leu Glu Ser Val Val Ile Leu Ser Thr Ala Ile Tyr Lys Thr Tyr Leu Ser Ile Arg   2539

7981  CGA GGC AAA AGT GAT GGT CTG CTA GGT ACA GGG GTT AGC GCG GCC ATG GAA ATT ATG TCA CAA AAC CCA GTA TCT GTG GGT ATA   8064
2540  Arg Gly Lys Ser Asp Gly Leu Leu Gly Thr Gly Val Ser Ala Ala Met Glu Ile Met Ser Gln Asn Pro Val Ser Val Gly Ile   2567

8065  GCA GTT ATG CTA GGG GTA GGG GCT GTA GCA GCC CAC AAT GCA ATT GAA GCC AGT GAG CAA AAA AGA ACA CTA CTT ATG AAA GTC   8148
2568  Ala Val Met Leu Gly Val Gly Ala Val Ala Ala His Asn Ala Ile Glu Ala Ser Glu Gln Lys Arg Thr Leu Leu Met Lys Val   2595
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8149  TTT GTG AAA AAC TTC TTA GAC CAG GCC GCC ACC GAC GAA CTA GTC AAA GAG AGC CCT GAG AAA ATA ATA ATG GCT TTG TTC GAA
2596  Phe Val Lys Asn Phe Leu Asp Gln Ala Ala Thr Asp Glu Leu Val Lys Glu Ser Pro Glu Lys Ile Ile Met Ala Leu Phe Glu   26.

8233  GCG GTG CAA ACG GTG GGC AAC CCT CTT AGA TTA GTG TAC CAC CTC TAT GGA GTT TTC TAT AAA GGG TGG GAA GCA AAA GAG TTG   8316
2624  Ala Val Gln Thr Val Gly Asn Pro Leu Arg Leu Val Tyr His Leu Tyr Gly Val Phe Tyr Lys Gly Trp Glu Ala Lys Glu Leu   2651

8317  GCC CAA AGA ACA GCC GGC AGG AAC CTT TTC ACC TTG ATA ATG TTC GAG GCT GTG GAA CTA CTG GGA GTA GAC AGT GAG GGA AAA   8400
2652  Ala Gln Arg Thr Ala Gly Arg Asn Leu Phe Thr Leu Ile Met Phe Glu Ala Val Glu Leu Leu Gly Val Asp Ser Glu Gly Lys   2679

8401  ATT CGC CAG CTA TCG AGC AAT TAC ATA CTA GAG CTC TTG TAT AAG TTC CGC GAC AAT ATC AAG TCT AGT GTG AGG GAG ATA GCA   8484
2680  Ile Arg Gln Leu Ser Ser Asn Tyr Ile Leu Glu Leu Leu Tyr Lys Phe Arg Asp Asn Ile Lys Ser Ser Val Arg Glu Ile Ala   2707

8485  ATC AGC TGG GCC CCC GCC CCC TTT AGT TGC GAT TGG ACA CCA ACA GAT GAC AGA ATA GGG CTT CCC CAT GAC AAT TAC CTC CGA   8568
2708  Ile Ser Trp Ala Pro Ala Pro Phe Ser Cys Asp Trp Thr Pro Thr Asp Asp Arg Ile Gly Leu Pro His Asp Asn Tyr Leu Arg   2735

8569  GTG GAG ACA AAG TGC CCC TGT GGT TAC AGG ATG AAA GCG GTA AAA AAC TGC GCT GGG GAG TTG AGA CTT CTG GAG GAA GGG GGT   8652
2736  Val Glu Thr Lys Cys Pro Cys Gly Tyr Arg Met Lys Ala Val Lys Asn Cys Ala Gly Glu Leu Arg Leu Leu Glu Glu Gly Gly   2763

8653  TCA TTC CTC TGC AGA AAT AAA TTC GGT AGA GGC TCA CAA AAC TAC AGG GTG ACA AAA TAC TAT GAT GAC AAT TTA TCA GAA ATA   8736
2764  Ser Phe Leu Cys Arg Asn Lys Phe Gly Arg Gly Ser Gln Asn Tyr Arg Val Thr Lys Tyr Tyr Asp Asp Asn Leu Ser Glu Ile   2791

8737  AAA CCA GTG ATA AGA ATG GAA GGA CAC GTG GAA CTG TAT TAC AAG GGG GCC ACT ATC AAA CTG GAT TTT AAC AAC AGT AAA ACG   8820
2792  Lys Pro Val Ile Arg Met Glu Gly His Val Glu Leu Tyr Tyr Lys Gly Ala Thr Ile Lys Leu Asp Phe Asn Asn Ser Lys Thr   2819

8821  GTA CTG GCA ACT GAC AAA TGG GAG GTT GAC CAC TCC ACC CTG GTT AGG GCA CTC AAG AGG TAC ACA GGG GCT GGA TAT CGA GGG   8904
2820  Val Leu Ala Thr Asp Lys Trp Glu Val Asp His Ser Thr Leu Val Arg Ala Leu Lys Arg Tyr Thr Gly Ala Gly Tyr Arg Gly   2847

8905  GCG TAT TTG GGT GAG AAA CCT AAC CAT AAA CAT CTG ATA CAG AGA GAC TGT GCA ACG ATT ACC AAA GAC AAG GTC TGC TTC ATC   8988
2848  Ala Tyr Leu Gly Glu Lys Pro Asn His Lys His Leu Ile Gln Arg Asp Cys Ala Thr Ile Thr Lys Asp Lys Val Cys Phe Ile   2875

8989  AAA ATG AAG AGA GGG TGT GCG TTC ACT TAT GAC CTA TCC CTC CAC AAC CTT ACC CGG CTA ATC GAA TTG GTA CAC AAG AAT AAC   9072
2876  Lys Met Lys Arg Gly Cys Ala Phe Thr Tyr Asp Leu Ser Leu His Asn Leu Thr Arg Leu Ile Glu Leu Val His Lys Asn Asn   2903

9073  CTG GAA GAT AGA GAA ATC CCT GCT GTG ACG GTT ACA ACC TGG CTG GCC TAC ACA TTT GTG AAT GAA GAC ATA GGG ACC ATA AAA   9156
2904  Leu Glu Asp Arg Glu Ile Pro Ala Val Thr Val Thr Thr Trp Leu Ala Tyr Thr Phe Val Asn Glu Asp Ile Gly Thr Ile Lys   2931

9157  CCA ACT TTT GGG GAA AAG GTG ACA CCG GAG AAA CAG GAG GAG GTA GTC TTG CAG CCT GCT GTG GTG GTG GAC ACA ACA GAT GTA   9240
2932  Pro Thr Phe Gly Glu Lys Val Thr Pro Glu Lys Gln Glu Glu Val Val Leu Gln Pro Ala Val Val Val Asp Thr Thr Asp Val   2959
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9241  GCC GTG ACC GTG GTA GGG GAA ACC TCT ACT ATG ACT ACA GGG GAG ACC CCG ACA ACA TTT ACC AGC TTA GGT TCG GAC TCG AAG   9324
2960  Ala Val Thr Val Val Gly Glu Thr Ser Thr Met Thr Thr Gly Glu Thr Pro Thr Thr Phe Thr Ser Leu Gly Ser Asp Ser Lys   2987

9325  GTC CGA CAA GTC CTG AAG CTG GGC GTG GAC GAT GGT CAA TAC CCC GGG CCT AAT CAG CAG AGA GCA AGC CTG CTC GAA GCT ATA   9408
2988  Val Arg Gln Val Leu Lys Leu Gly Val Asp Asp Gly Gln Tyr Pro Gly Pro Asn Gln Gln Arg Ala Ser Leu Leu Glu Ala Ile   3015

9409  CAA GGT GTG GAT GAA AGG CCC TCG GTA CTG ATA CTG GGG TCT GAT AAG GCC ACC TCC AAT AGG GTC AAG ACC GCA AAG AAT GTG   9492
3016  Gln Gly Val Asp Glu Arg Pro Ser Val Leu Ile Leu Gly Ser Asp Lys Ala Thr Ser Asn Arg Val Lys Thr Ala Lys Asn Val   3043

9493  AAG ATA TAT AGG AGC AGG GAC CCC CTG GAA CTG AGA GAA ATG ATG AAA AGG GGA AAA ATC CTA GTC GTA GCC TTG TCT AGA GTC   9576
3044  Lys Ile Tyr Arg Ser Arg Asp Pro Leu Glu Leu Arg Glu Met Met Lys Arg Gly Lys Ile Leu Val Val Ala Leu Ser Arg Val   3071

9577  GAT ACC GCT CTG CTG AAA TTC GTT GAT TAC AAA GGC ACC TTC CTG ACC AGA GAG ACC CTA GAG GCA TTA AGT CTG GGT AAG CCT   9660
3072  Asp Thr Ala Leu Leu Lys Phe Val Asp Tyr Lys Gly Thr Phe Leu Thr Arg Glu Thr Leu Glu Ala Leu Ser Leu Gly Lys Pro   3099

9661  AAG AAA AGA GAC ATA ACT AAA GCA GAA GCA CAA TGG CTG CTG CGC CTC GAA GAC CAA ATA GAA GAG CTG CCT GAC TGG TTC GCA   9744
3100  Lys Lys Arg Asp Ile Thr Lys Ala Glu Ala Gln Trp Leu Leu Arg Leu Glu Asp Gln Ile Glu Glu Leu Pro Asp Trp Phe Ala   3127

9745  GCC AAG GAA CCC ATA TTT CTA GAA GCC AAC ATT AAA CGT GAC AAG TAT CAC CTG GTA GGG GAC ATA GCC ACT ATT AAA GAA AAA   9828
3128  Ala Lys Glu Pro Ile Phe Leu Glu Ala Asn Ile Lys Arg Asp Lys Tyr His Leu Val Gly Asp Ile Ala Thr Ile Lys Glu Lys   3155

9829  GCC AAA CAA CTG GGG GCA ACA GAC TCC ACA AAG ATA TCA AAG GAG GTT GGC GCG AAA GTG TAT TCT ATG AAG CTG AGT AAC TGG   9912
3156  Ala Lys Gln Leu Gly Ala Thr Asp Ser Thr Lys Ile Ser Lys Glu Val Gly Ala Lys Val Tyr Ser Met Lys Leu Ser Asn Trp   3183

9913  GTG ATA CAA GAA GAG AAT AAA CAA GGC AGC CTT GCC CCC CTG TTT GAA GAG CTC CTG CAA CAG TGC CCA CCC GGG GGC CAG AAC   9996
3184  Val Ile Gln Glu Glu Asn Lys Gln Gly Ser Leu Ala Pro Leu Phe Glu Glu Leu Leu Gln Gln Cys Pro Pro Gly Gly Gln Asn   3211

9997  AAA ACC ACA CAT ATG GTC TCA GCC TAC CAA CTA GCT CAA GGG AAT TGG GTG CCA GTT AGT TGC CAC GTG TTC ATG GGG ACC ATA   10080
3212  Lys Thr Thr His Met Val Ser Ala Tyr Gln Leu Ala Gln Gly Asn Trp Val Pro Val Ser Cys His Val Phe Met Gly Thr Ile   3239

10081 CCC GCC AGA AGA ACC AAG ACT CAT CCT TAT GAG GCA TAC GTT AAG CTA AGG GAG TTG GTA GAT GAA CAT AAG ATG AAG GCA TTA   10164
3240  Pro Ala Arg Arg Thr Lys Thr His Pro Tyr Glu Ala Tyr Val Lys Leu Arg Glu Leu Val Asp Glu His Lys Met Lys Ala Leu   3267

10165 TGT GGC GGA TCA GGC CTA AGT AAG CAC AAC GAA TGG GTA ATT GGC AAG GTC AAG TAT CAA GGA AAC CTG AGG ACC AAA CAC ATG   10248
3268  Cys Gly Gly Ser Gly Leu Ser Lys His Asn Glu Trp Val Ile Gly Lys Val Lys Tyr Gln Gly Asn Leu Arg Thr Lys His Met   3295
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10249  TTG AAC CCC GGA AAG GTG GCG GAG CAA CTG CAC AGA GAA GGG TAC AGG CAC AAT GTG TAT AAT AAG ACA ATA GGT TCA GTG ATG   10332
 3286  Leu Asn Pro Gly Lys Val Ala Glu Gln Leu His Arg Glu Gly Tyr Arg His Asn Val Tyr Asn Lys Thr Ile Gly Ser Val Met   3323

10333  ACA GCA ACT GGT ATC AGG CTG GAG AAG TTA CCT GTG GTT AGG GCC CAA ACA GAC ACA ACC AAC TTC CAC CAA GCA ATA AGG GAT   10416
 3324  Thr Ala Thr Gly Ile Arg Leu Glu Lys Leu Pro Val Val Arg Ala Gln Thr Asp Thr Thr Asn Phe His Gln Ala Ile Arg Asp   3351

10417  AAA ATA GAC AAG GAG GAG AAC CTA CAG ACC CCT GGC TTG CAT AAG AAG TTA ATG GAA GTC TTC AAT GCA TTA AAA AGA CCC GAG   10500
 3352  Lys Ile Asp Lys Glu Glu Asn Leu Gln Thr Pro Gly Leu His Lys Lys Leu Met Glu Val Phe Asn Ala Leu Lys Arg Pro Glu   3379

10501  CTT GAG GCC TCT TAT GAC GCT GTG GAT TGG GAG GAA TTG GAG AGA GGA ATA AAT AGG AAG GGT GCT GCT GGT TTC TTC GAA CGC   10584
 3380  Leu Glu Ala Ser Tyr Asp Ala Val Asp Trp Glu Glu Leu Glu Arg Gly Ile Asn Arg Lys Gly Ala Ala Gly Phe Phe Glu Arg   3407

10585  AAG AAC ATA GGA GAG GTT TTG GAT TCG GAA AAA AAT AAA GTC GAA GAG GTT ATT GAC AGT TTG AAA AAA GGT AGG AAT ATC AGA   10668
 3408  Lys Asn Ile Gly Glu Val Leu Asp Ser Glu Lys Asn Lys Val Glu Glu Val Ile Asp Ser Leu Lys Lys Gly Arg Asn Ile Arg   3435

10669  TAC TAC GAA ACT GCA ATC CCG AAA AAC GAG AAG AGG GAT GTC AAT GAT GAC TGG ACC GCT GGT GAC TTC GTA GAT GAG AAG AAG   10752
 3436   Tyr Tyr Glu Thr Ala Ile Pro Lys Asn Glu Lys Arg Asp Val Asn Asp Asp Trp Thr Ala Gly Asp Phe Val Asp Glu Lys Lys   3463

10753  CCA AGA GTG ATA CAA TAC CCT GAG GCT AAA ACT AGG TTG GCT ATT ACT AAG GTA ATG TAC AAG TGG GTC AAA CAG AAG CCA GTT   10836
 3464  Pro Arg Val Ile Gln Tyr Pro Glu Ala Lys Thr Arg Leu Ala Ile Thr Lys Val Met Tyr Lys Trp Val Lys Gln Lys Pro Val   3491

10837  GTC ATA CCG GGT TAT GAA GGT AAG ACA CCC CTG TTT CAA ATT TTT GAC AAA GTG AAG AAA GAA TGG GAT CAA TTC CAA AAC CCT   10920
 3492  Val Ile Pro Gly Tyr Glu Gly Lys Thr Pro Leu Phe Gln Ile Phe Asp Lys Val Lys Lys Glu Trp Asp Gln Phe Gln Asn Pro   3519

10921  GTG GCA GTT AGC TTT GAT ACC AAA GCG TGG GAT ACC CAG GTA ACC ACA AGG GAT TTG GAG CTA ATA AGG GAT ATA CAG AAG TTC   11004
 3520  Val Ala Val Ser Phe Asp Thr Lys Ala Trp Asp Thr Gln Val Thr Thr Arg Asp Leu Glu Leu Ile Arg Asp Ile Gln Lys Phe   3547
                                                                                  Ser

11005  TAT TTT AAA AAG AAA TGG CAC AAA TTC ATT GAC ACC CTA ACC AAG CAC ATG TCA GAA GTA CCC GTA ATC AGT GCC GAC GGG GAG   11088
 3548  Tyr Phe Lys Lys Lys Trp His Lys Phe Ile Asp Thr Leu Thr Lys His Met Ser Glu Val Pro Val Ile Ser Ala Asp Gly Glu   3575

11089  GTA TAC ATA AGG AAA GGT CAG AGA GGC AGT GGG CAA CCT GAC ACG AGC GCA GGC AAC AGC ATG TTG AAT GTG TTG ACA ATG GTG   11172
 3576  Val Tyr Ile Arg Lys Gly Gln Arg Gly Ser Gly Gln Pro Asp Thr Ser Ala Gly Asn Ser Met Leu Asn Val Leu Thr Met Val   3603

11173  TAT GCC TTC TGC GAG GCC ACG GGG GTA CCC TAC AAG AGT TTT GAC AGA GTG GCA AAG ATC CAT GTC TGC GGG GAT GAT GGT TTC   11235
 3604  Tyr Ala Phe Cys Glu Ala Thr Gly Val Pro Tyr Lys Ser Phe Asp Arg Val Ala Lys Ile His Val Cys Gly Asp Asp Gly Phe   3631
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11257  CTG ATT ACC GAA AGA GCT CTC GGT GAG AAA TTT GCG AGT AAA GGA GTC CAG ATC CTA TAC GAA GCT GGG AAG CCT CAA AAG ATC   11340
 3632  Leu Ile Thr Glu Arg Ala Leu Gly Glu Lys Phe Ala Ser Lys Gly Val Gln Ile Leu Tyr Glu Ala Gly Lys Pro Gln Lys Ile   3659

11341  ACT GAA GGG GAC AAG ATG AAA GTA GCC TAT CAG TTT GAT GAT ATC GAG TTC TGC TCC CAT ACA CCA GTA CAA GTG AGG TGG TCA   11424
 3660  Thr Glu Gly Asp Lys Met Lys Val Ala Tyr Gln Phe Asp Asp Ile Glu Phe Cys Ser His Thr Pro Val Gln Val Arg Trp Ser   3687

11425  GAC AAT ACT TCC AGC TAC ATG CCG GGA AGG AAC ACG ACT ACA ATC CTG GCT AAA ATG GCT ACA AGG TTG GAT TCC AGT GGT GAG   11508
 3688  Asp Asn Thr Ser Ser Tyr Met Pro Gly Arg Asn Thr Thr Thr Ile Leu Ala Lys Met Ala Thr Arg Leu Asp Ser Ser Gly Glu   3715

11509  AGG GGT ACT ATA GCA TAT GAG AAG GCA GTG GCG TTC AGC TTT TTG TTG ATG TAC TCC TGG AAC CCA CTG ATC AGA AGG ATA TGC   11592
 3716  Arg Gly Thr Ile Ala Tyr Glu Lys Ala Val Ala Phe Ser Phe Leu Leu Met Tyr Ser Trp Asn Pro Leu Ile Arg Arg Ile Cys   3743

11593  TTA CTG GTG TTG TCA ACT GAG TTG CAA GTG AGA CCA GGG AAG TCA ACC ACC TAT TAC TAT GAA GGG GAC CCA ATA TCC GCT TAC   11676
 3744  Leu Leu Val Leu Ser Thr Glu Leu Gln Val Arg Pro Gly Lys Ser Thr Thr Tyr Tyr Tyr Glu Gly Asp Pro Ile Ser Ala Tyr   3771

11677  AAG GAA GTC ATT GGC CAC AAT CTC TTT GAC CTT AAA AGA ACA AGC TTC GAA AAG CTA GCA AAG TTA AAT CTC AGC ATG TCC ACG   11760
 3772  Lys Glu Val Ile Gly His Asn Leu Phe Asp Leu Lys Arg Thr Ser Phe Glu Lys Leu Ala Lys Leu Asn Leu Ser Met Ser Thr   3799

                                                                    !
11761  CTC GGG GTG TGG ACT AGA CAC ACT AGC AAG AGA TTA CTA CAA GAT TGT GTC AAT GTT GGC ACC AAA GAG GGC AAC TGG CTG GTC   11844
 3800  Leu Gly Val Trp Thr Arg His Thr Ser Lys Arg Leu Leu Gln Asp Cys Val Asn Val Gly Thr Lys Glu Gly Asn Trp Leu Val   3827

11845  AAT GCA GAC AGA CTA GTG AGT AGT AAG ACA GGA AAC AGG TAT ATA CCT GGA GAG GGC CAC ACC CTA CAA GGG AAA CAT TAT GAA   11928
 3828  Asn Ala Asp Arg Leu Val Ser Ser Lys Thr Gly Asn Arg Tyr Ile Pro Gly Glu Gly His Thr Leu Gln Gly Lys His Tyr Glu   3855

11929  GAA CTG ATA CTG GCA AGG AAA CCG ATC GGT AAC TTT GAA GGG ACC GAT AGG TAT AAC TTG GGG CCA ATA GTC AAT GTA GTG TTG   12012
 3856  Glu Leu Ile Leu Ala Arg Lys Pro Ile Gly Asn Phe Glu Gly Thr Asp Arg Tyr Asn Leu Gly Pro Ile Val Asn Val Val Leu   3883

12013  AGG AGA CTA AAA ATT ATG ATG ATG GCC CTG ATA GGA AGG GGG GTG TGA GCA TGG TTG GCC CTT GAT CGG GCC CTA TCA GTA GAA   12096
 3884  Arg Arg Leu Lys Ile Met Met Met Ala Leu Ile Gly Arg Gly Val End                                                  3899

12097  CCC TAT TGT AAA TAA CAT TAA CTT ATT AAT TAT TTA GAT ACT ATT ATT TAT TTA TTT ATT TAT TTA TTG AAT GAG CAA GTA CTG   12180

12181  GTA CAA ACT ACC TCA TGT TAC CAC ACT ACA CTC ATT TTA ACA GCA CTT TAG CTG GAG GGA AAA CCC TGA CGT CCA CAG TTG GAC   12264

12265  TAA GGT AAT TTC CTA ACG GC 12284
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Figure 3. Nucleotide sequence.

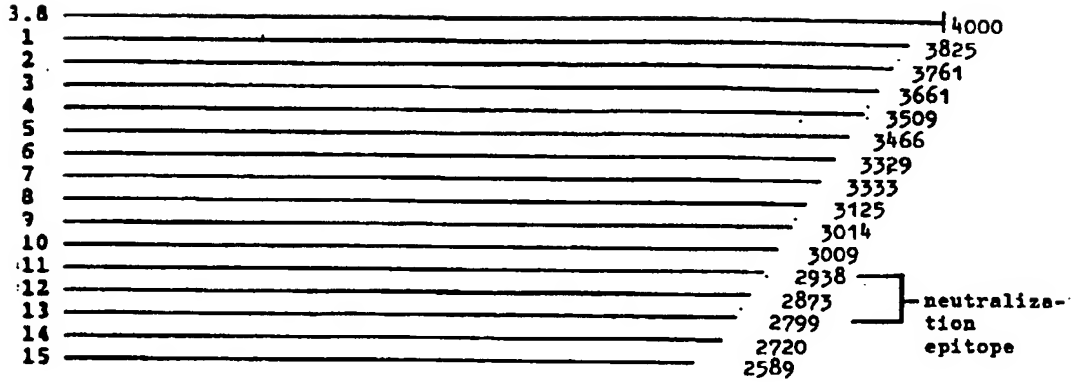
HCV

AGTGACAACGGCACTAATGGTATTCAGCGAGCCATGTATCTTAGAGGGGTTAACAGG
AGCTTACATGGGATCTGGCCCGAGAAAATATGCAAGGGGGTCCCCACTCATCTGGCC
ACTGACACGGAACTGAAAGAGATACGCGGGATGATGGATGCCAGCGAGAGGACAAAC
TATACGTGCTGTAGGTTACAAAGACATGAATGGAACAAACATGGATGGTGTAACTGG
TACAACATAGACCCTTGGATTCAGTTAATGAACAGGACCCAAACAAATTTGACAGAA
GGCCCTCCAGATAAG

Deduced amino acid sequence.

HCV	S	D	N	G	T	N	G	I	Q	R	A	M	Y	L	R	G	V	N	R	S
	L	H	G	I	W	P	E	K	I	C	K	G	V	P	T	H	L	A	T	D
	T	E	L	K	E	I	R	G	M	M	D	A	S	E	R	T	N	Y	T	C
	C	R	L	Q	R	H	E	W	N	K	H	G	W	C	N	W	Y	N	I	D
	P	W	I	Q	L	M	N	R	T	Q	T	N	L	T	E	G	P	P	D	K

Figure 4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3661
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2589
neutralization epitope

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(54) Hog cholera virus vaccine and diagnostic.

(57) The present invention is concerned with a hog cholera virus vaccine comprising a polypeptide characteristic of hog cholera virus. Vector vaccines capable to express a nucleic acid sequence encoding such a polypeptide also form part of the present invention. Said polypeptide and nucleic acid sequence can also be used for the detection of hog cholera virus infection.

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